The effect of environmental and social factors on the courtship and mating dynamics of the smooth newt, Triturus v. vulgaris

Thesis

The Effect of Environmental and Social Factors on the Courtship and Mating Dynamics of the Smooth Newt, *Triturus v. vulgaris*

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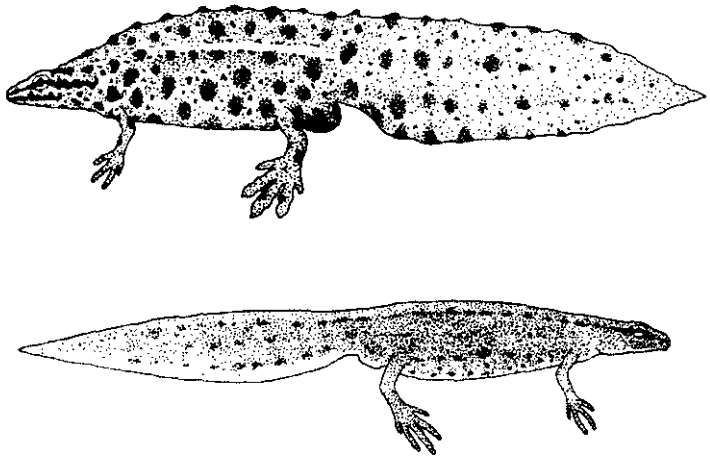

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Abstract

The effect of environmental and social factors on the courtship and mating dynamics of the smooth newt, *Triturus v. vulgaris*, was studied using laboratory experiments, an individual-based model, a semi-natural population and a field study. In view of the limitations of laboratory experiments and field observation, the semi-natural and modelling approaches are recommended as additional research tools.

In the laboratory, the optimal temperature for spermatophore transfer was just below 13°C, at which point the spermatophore deposition rate is high and oxygen availability not limiting. In the wild females determine the timing of mating. They are highly receptive for a brief period after arrival at the breeding site (early spring), subsequently re-mating only sporadically. Therefore, few courtships take place at mid-season, optimal temperatures.

In the semi-natural population, deposition occurred in only 3 – 6% of courtships with a median of one deposition per encounter. Male mating success is therefore unlikely to be constrained by physiological capacity for spermatophore production. Female receptivity and immigration patterns are thus the principle determinants of the operational sex ratio (OSR). According to the model, a male-biased OSR develops rapidly at the start of the season, but the strength of bias will depend on the breeding sex ratio, duration of the arrival period and extent to which males arrive before females.

The OSR influences the intensity of competition for mates and the potential for sexual selection. Males compete directly for females by sexual interference, a common but low-gain strategy. Males may also compete indirectly by courting unmated and unfamiliar females preferentially (both of which have a higher probability of being receptive) although more research is needed to establish if males differ in their ability to find receptive females. There is variance in male mating success but the relative contributions of environmental factors and phenotypic traits needs further clarification.

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Introduction

1.1 General introduction

Our understanding of the courtship and mating dynamics of urodeles lags behind that of anuran amphibians and other vertebrate taxa. Many species are cryptic or live in inaccessible habitats and thus pose considerable problems for observation in the wild. In his review of urodelan sexual strategies, Verrell (1989a) drew attention to the gap between the numerous laboratory studies and the limited field data available for these amphibians. More recently, these limitations have been reiterated (Sullivan et al. 1995; Halliday 1998).

The smooth newt (*Triturus v. vulgaris*) provides a good example of a species which is difficult to observe in its natural habitat. During the spring, newts migrate from terrestrial hiding places to breed in murky, weed-choked ponds. As a result, laboratory studies have dominated the intensive investigation into the reproductive behaviour of this species over the last twenty-five years (for example, Halliday 1974; 1976; Harrison et al. 1983; Verrell 1984a; Griffiths 1985; Verrell and Halliday 1985a; Verrell and McCabe 1988; Halliday 1990; Green 1991a; Baker 1992a; Hosie 1992; Pecio 1992; Cogãlniceanu 1994; Waights 1996; Gabor and Halliday 1997). Laboratory experiments, however, tend to reduce the complexity of both the physical and social environment in which the animals usually behave. For example, courtship has often been described from a single pair of newts in a simple aquarium. Little is known, therefore, about the influence of environmental and social factors on the courtship and mating dynamics of this species.

1.1.1 Temperature

Verrell (1989a) and later Halliday (1992) emphasised the importance of understanding the constraints imposed by physiological capabilities on the sexual behaviour of smooth newts and there has been subsequent research into the cost of spermatophore production and crest development (Green 1991a; 1991b; Baker 1992b; Sever et al. in press). However, the relationship between the *physical environment*, physiological capabilities and sexual behaviour

has not been investigated. Over the last twenty-five years, an interest in studying animal physiology in the context of the natural environment has resulted in the discipline of *Environmental Physiology* (Feder 1992). In their extensive review, Feder and Burggren (1992) clearly demonstrate the importance of the environment to the physiology and behaviour of amphibians. However, they also point out the paucity and inconsistency of much of the available data, particularly with regard to urodeles.

Temperature is one of the most pervasive physical influences on animal life. Through its effect on the biochemical reactions that underlie physiological processes, it has a wide-ranging influence from geographical distribution to enzyme function (Prosser 1986). In relation to their response to the thermal environment, animals can be described as poikilotherms (body temperature fluctuating with the surrounding environment) or homeotherms (maintaining a near constant body temperature) and ectotherms (heat gained from an external source, primarily solar radiation) or endotherms (heat produced by the internal consumption of energy) (Huey 1982).

Most amphibians are both poikilothermic and ectothermic and many species inhabit a variable environment in which temperature exerts a strong influence at a variety of temporal scales (Table 1.1).

Time scale	**Influence of temperature**	**Reference**
Evolutionary time	Geographical distribution	(Brattstrom 1979; Prosser 1991)
Years	Life history, for example growth and development	(Berven 1981; Dunham et al. 1989; Atkinson 1994)
Seasons	Spermiogenesis, embryonic development	(Bachmann 1969; Fraile et al. 1989)
Days	Food passage time	(Jiang and Claussen 1993)
Hours and minutes	Locomotion, breathing patterns, metabolic rate, heart rate	(Rome et al. 1992)

Table 1.1. Examples of the influence of temperature on amphibians at a range of temporal scales.

Sexual activity in the smooth newt takes place in the spring and summer, during which time the temperature of the water may vary by as much as 30°C (Chapter three). I decided to

investigate the effect of temperature on sexual activity both over a short timescale (a courtship encounter) and over the whole season, using controlled laboratory experiments and a longitudinal study of a semi-natural population.

1.1.2 Social factors

The operational sex ratio or 'the average ratio of fertilizable females to sexually active males at any given time' is closely related to the intensity of competition for mates, variance in mating success and the opportunity for sexual selection (Emlen and Oring 1977). Within the constraints of the physical environment, mating success is thus determined by the social environment and the outcome of interactions between individuals.

In the smooth newt, the operational sex ratio (the ratio of males with spermatophores to receptive females: section 1.2.1 below) is difficult to measure accurately in the field. There is some evidence that the operational sex ratio varies during the breeding season (Verrell and McCabe 1988) but more information is needed to clarify the extent of variation, the principle determinants of the OSR and the implications for the operation of sexual selection.

Sperm transfer in smooth newts is not only inherently unreliable (section 1.2.1 below) but males are particularly vulnerable to competition via sexual interference (Arnold 1976; Halliday 1990; Chapter four). Although sexual interference in the smooth newt has been described from laboratory studies (Verrell 1984a) the effect it has on mating success and the prevalence of the behaviour in the wild is poorly known (Verrell 1989a; Sullivan et al. 1995). There may be additional ways in which the presence of other individuals affects sexual activity. Verrell (1983), for example, showed that the sexual behaviour of male *Notophthalmus viridescens* (Salamandridae) was influenced by the number of available females.

Measurement of mating success requires both individual recognition and a longitudinal study and, to my knowledge, has not been undertaken for any species of *Triturus*. Social

factors were investigated using a semi-natural population, experiments involving groups of newts, a field study and an individual-based model.

1.2 Reproduction in the smooth newt

1.2.1 The breeding season

The breeding period of the smooth newt begins in early spring (February/March in Great Britain) with migration from the terrestrial habitat to an aquatic breeding site (Harrison et al. 1983; Verrell and Halliday 1985a). There is considerable variability in the breeding sex ratio, the duration of the arrival period and the extent to which males arrive before females (Chapter six). Newts adapt to the aquatic phase with a number of anatomical and physiological changes including modification of the skin to improve respiration under water and development of the lateral-line organs (Halliday 1974).

Courtship in the genus *Triturus* is characterised by two features. First, the sexes are sexually dimorphic during the breeding season, the males developing striking secondary sexual characters (1977a). In *T. vulgaris*, males grow a deep crest which extends to the base of the tail and develop fringes of skin around the toes of the hindlimb. The body, tail and crest may be conspicuously marked with spots and blotches, the belly becomes bright orange, the head striped and the tip of the tail is marked with orange and silver stripes. Females are greenish-brown with a pale to orange belly and variable spotting (Halliday 1974).

Second, there is no form of physical restraint (amplexus) during courtship (Halliday 1977a); instead the female is stimulated by male display (see below). Sperm transfer occurs externally via a spermatophore, consisting of a sperm mass and gelatinous base. The sperm mass is absorbed by the female and sperm migrate to the spermathecae or storage tubules (Halliday and Verrell 1984; Halliday 1998) where they remain in a quiescent state (Sever et al. in press). Internal fertilisation occurs during oviposition when sperm are released on to the

eggs as they migrate through the cloaca (Sever et al. in press). In the laboratory, egg-laying begins between two and ten days after mating (Verrell 1984b; Hosie 1992; Pecio 1992).

The eggs (less than 2mm in diameter) are laid singly (Bell and Lawton 1975) each one wrapped in weed for protection against predators (Miaud 1994). Clutch size is positively correlated with body size and females lay between 100 and 600 eggs per season (Verrell et al. 1986; Baker 1992a). This may take several weeks and egg-laying has been reported to peak in the wild in April and May (Bell and Lawton 1975; Verrell and McCabe 1988; Chapter three).

The eggs hatch in about 20 days (at 17°C) and larvae remain in the pond until metamorphosis occurs, usually between July and September (Bell and Lawton 1975). The emerging efts (terrestrial metamorphs) remain on land until they reach sexual maturity (Bell 1977). Most adults also leave the pond at the end of the summer (Harrison et al. 1983; Verrell and Halliday 1985a; Verrell and McCabe 1988) and spend the winter months in terrestrial refugia (Griffiths 1984).

1.2.2 Courtship

The terms used in this thesis follow Halliday's (1974) description of smooth newt courtship. A brief overview is given here. A sexual encounter begins with a directed approach by the male or female, or following chance contact between individuals (Halliday 1974; Hosie 1992). After a period of sniffing and chasing, the male attempts to take up his display position with a *'move-to-the-front'*. The male swims to the front of the female using a powerful tail movement and stops perpendicular to her snout so that she is prevented from moving forward. The female, nevertheless, frequently pushes her way past the male and several cycles of chasing and move-to-the-front may occur before the male can continue with display. This is known as the *orientation phase* (Figure 1.1).

In the next part of the sequence, the male uses a series of tail movements to stimulate the female. The *whip* is a violent lash of the tail (similar to the movement which ends move-to-

the-front) which sends a blast of water towards the female's head. *Fanning* consists of a gentle, sustained tail vibration which directs a steady movement of water towards the female. *Wave* describes a more variable set of movements including twitching the tip of the tail. During these movements, the male faces the female, positioned two to five centimetres in front of her snout. Since 'wave' encompasses more than one action, there tends to be low inter-observer reliability for wave scores. Fan and whip scores, however, give adequate measures of male performance (Halliday 1976) and wave has therefore been omitted from the analysis of display in this thesis.

The order and number of these display acts are controlled primarily by the female. If the female moves away, the male attempts to resume display in front of her. If the female remains stationary, the male exhibits *static display*, alternating between tail movements in a variable manner. Eventually the female will start to approach the male, causing him to retreat before her while displaying. Once *retreat display* has begun, the female usually continues to approach, encouraging the male to enter the *spermatophore transfer phase* (Figure 1.1). Highly motivated females tend to approach from the start, eliminating the period of static display altogether.

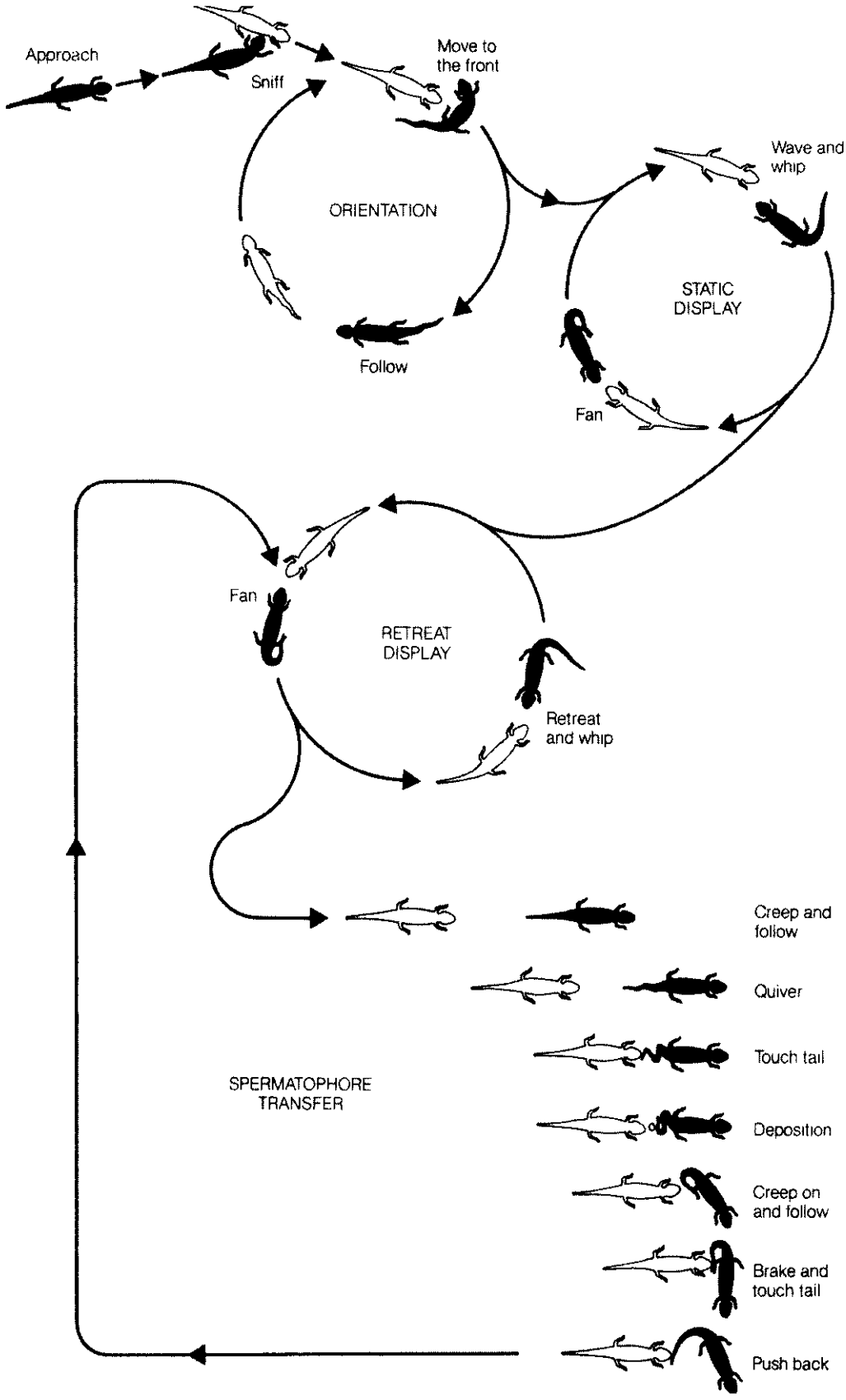


Figure 1.1. Diagram of courtship display during a single encounter. The male is in black. Source: Halliday (1974).

The spermatophore transfer phase (the most stereotyped part of courtship) begins when the male turns away from the female and goes into *creep.* He moves forward, in front of the female with his tail held straight. After a short distance, the male stops in *quiver* and waits for the female while his tail continues to move in rippling waves. The female continues to approach the male until she nudges the tip of his tail. This *tail-touch* is the signal for the male to fold and lift his tail allowing him to deposit a spermatophore on to the substrate. The male then moves forward about one body length and turns at an angle of 90° into *brake.* The female, still following closely behind, stops as she meets the male's flank, whereupon her cloaca is approximately positioned over the spermatophore.

The spermatophore may adhere to the female's cloaca, allowing the sperm mass to be gradually absorbed into the spermathecae. On many occasions, however, *pick-up* of the spermatophore by the female is unsuccessful and possible explanations for the failure to pick up are discussed in the following chapters. The spermatophore transfer phase marks the end of a single *sequence.* Subsequently, the pair either separate or the male attempts to resume display. If the female is still receptive, the process is repeated until a second spermatophore transfer phase is completed. A courtship *encounter* may contain several sequences.

Visual, olfactory and tactile stimuli are all important during courtship in *Triturus* (Halliday 1974; Himstedt 1979; Verrell 1986a; Belvedere et al. 1988; Hosie 1992; Cogãlniceanu 1994) Halliday (1974) suggested that each part of the male's display in *T. vulgaris* provides a different stimulus. The wave allows a clear view of the male's flank and crest, while the whip send a powerful blast of water towards the female which may be sensed by her lateral line organs. Fanning produces a current of water that directs pheromones from the male's dorsal gland towards the female's snout.

According to Sullivan et al. (1995) the dorsal gland of male *T. vulgaris* may represent almost 10% of body weight at the height of the season. Courtship pheromones (containing

progesterone) are thought to act as a sexual attractant to both males and females (Belvedere et al. 1988; Halliday 1990) and olfactory cues are reported to be particularly important during mate location and the early stages of courtship (Hosie 1992; Cogãlniceanu 1994) although in *T. alpestris*, the bright red belly has also been shown to be a stimulus for the initiation of courtship (Himstedt 1979).

1.2.3 Mating dynamics

The mating system of the smooth newt has been described (tentatively) as scramble competition polygyny which is 'typified by males actively searching for females and interacting with other males across wide areas of the breeding site' (Sullivan et al. 1995). This is appropriate insofar as males appear to move freely within the pond and compete for successive females through sexual interference (Verrell 1984a; Chapters four and five).

However, females also mate repeatedly in the laboratory (Verrell 1984a; 1984b; Hosie 1992) and it may be more useful to describe both sexes as polygamous. Multiple mating by females was once considered an enigma since the number of sperm gained in a single mating is usually more than sufficient to fertilise the entire egg supply (Halliday and Verrell 1984) but, more recently, a number of advantages have been put forward. Potential direct benefits to females include insuring or increasing fertility (Birkhead and Parker 1997; Hoogland 1998), avoiding genetic incompatibility (Jennions 1997; Hosken and Stockley 1998), nutrient acquisition, paternal care and the avoidance of harassment (Birkhead and Parker 1997). Indirect benefits include increasing genetic diversity (Greene and Brown 1991) or genetic quality (Birkhead and Parker 1997). Halliday and Arnold (1987; 1988; 1992) also proposed that multiple mating in females may have arisen as a result of genetic correlation with male mating success. Appropriate conditions for multiple mating by females arise in many species of urodele since the operational sex ratio is often male-biased, the mating period lasts several months and males continue to produce spermatophores over this period (Halliday and Verrell 1984; Halliday 1998).

Although newts may be present in the water for several months, a laboratory-based, longitudinal study of eight females suggested that female sexual motivation varies greatly throughout the breeding season (Hosie 1992). During the first six trials (about two weeks) female motivation to mate was high and mating occurred between one and three times in quick succession. Fifty percent of all observed inseminations occurred within this period. Females subsequently mated only once or twice more during the remainder of the season. This supported previous work (Halliday and Verrell 1984; Verrell 1984b) in which females were found to become unresponsive to males, following an initial intense period of mating and the onset of oviposition.

Male smooth newts have a dissociated breeding cycle (Crews 1987) in which spermatogenesis occurs during the summer and early autumn, all mature sperm production being completed before the beginning of the breeding season (Verrell et al. 1986). Males therefore have a finite spermatophore supply each season. It is also recognised that, in many species, ejaculates are costly to produce (Dewsbury 1982) and male sperm supply can be depleted temporarily, requiring a number of days for replenishment between ejaculates. There is evidence from laboratory experiments that male smooth newts are constrained by spermatophore production (Halliday 1998) both on a seasonal (Halliday 1976) and daily (Verrell 1986b) basis.

Female receptivity and male spermatophore production, together with the temporal distribution of the sexes and the adult sex ratio will determine the operational sex ratio (Clutton-Brock and Parker 1992). Since these factors are not constant, the OSR is likely to fluctuate during the breeding season. Verrell and McCabe (1988) proposed that, within a female-biased breeding sex ratio, a female-biased OSR may arise at the beginning of the season as a result of high female receptivity and temporary depletion of male spermatophore supplies. Following the onset of ovulation, female receptivity is reduced and the operational sex ratio becomes heavily male-biased. This simple scenario, however, does not take the

influence of immigration patterns into account (Chapter six). As mentioned above, the OSR affects the intensity of competition for mates, variance in mating success and the potential for sexual selection. If the OSR fluctuates over time, there may be temporal variation in the intensity of sexual selection (Verrell and McCabe 1988).

Multiple mating by females provides the potential for sperm competition or 'competition between ejaculates for ova' (Parker 1970; Birkhead and Parker 1997). Very little is known about the mechanism of sperm competition and its effect on male mating success in urodeles (Halliday and Verrell 1984; Halliday 1998) but this is currently the focus of intensive research (Sever et al. in press; Arano and Halliday unpubl. data). Nevertheless, it is clear that sperm competition precludes a direct correlation between mating success and reproductive success.

1.3 Thesis structure

The effect of temperature on courtship behaviour and breathing patterns is the subject of Chapter two. Controlled laboratory experiments are used to ask if there is an optimal temperature for spermatophore transfer. Chapter three presents data from a longitudinal study of a semi-natural population which examines the effect of temperature on sexual activity throughout the breeding season.

Chapter four re-examines the data from the semi-natural population in terms of sexual interference and individual mating success. Chapter five continues with the theme of social interactions between individuals, describing two experiments in which groups of newts are used to investigate how males obtain their mates. The first asks if males respond differently to familiar and unfamiliar females, while the second looks at the effect of male body size on mating success during sexual interference.

Chapters six and seven focus on the operational sex ratio. The variation in breeding sex ratio and arrival patterns is examined in Chapter six, while Chapter seven uses a computer simulation to investigate the effect of such variation on the operational sex ratio, variance in

mating success and the potential for sexual selection. Details of the model structure and of experiments used to test the assumptions of the model are given in the appendices.

Thus four principle questions are addressed in this thesis:-

1. What is the effect of temperature on courtship at both short-term and seasonal timescales?
2. What are the main factors determining the operational sex ratio?
3. How does the social environment affect individual mating success?
4. Is there variance in mating success and if so, what are the causes of such variance?

Chapter eight brings together the answers to these questions in an attempt to understand the influence of environmental and social factors on the courtship and mating dynamics of the smooth newt.

Chapter two

The effect of temperature: laboratory experiments

2.1 Introduction

This chapter is concerned with the effect of temperature on the courtship of the smooth newt in a controlled environment and asks whether there is an optimal temperature for spermatophore transfer. In thermal physiology the term 'optimal' is often used synonymously with 'maximal' to denote the temperature range at which performance peaks. Many authors acknowledge, however, that maximal physiological temperatures may not be optimal in a broader context (Huey 1982). In this chapter 'optimal' is used to denote the temperature at which spermatophore transfer is most successful. The next chapter discusses the validity of this approach in relation to a more natural environment.

In Great Britain, during the prolonged breeding season, smooth newts experience a wide range of temperatures from just above freezing to 30°C (Chapter three). Both extremes pose problems for their energetic courtship behaviour: at high temperatures, the increase in metabolic rate and oxygen consumption coincides with a decrease in the dissolved oxygen content of the water; at low temperatures, metabolic rate and muscular activity are depressed. Two important factors influencing spermatophore transfer in the smooth newt are the effectiveness of the male's display and the conflict between courtship and the need to breathe air. In this introduction, I discuss the effect of temperature on the respiration and locomotion of aquatic amphibians and apply this, where possible, to *Triturus vulgaris*.

Temperature is a measure of the average velocity of particles, an increase in temperature producing a corresponding increase in velocity. A rise in temperature usually causes an acceleration in the rate of biochemical processes (Rome et al. 1992). The change in the rate of a process with a 10°C change in temperature is known as the Q_{10}. A Q_{10} of between two and three for maximal and resting aerobic metabolism is common in many poikilothermic

vertebrates (Bennett 1980; Gatten et al. 1992). The Q_{10} is often greater at low temperatures (below 10°C), falling to about 1.5 as temperature rises to 30°C (Gatten et al. 1992).

A frequent approach to the study of physiological processes in relation to temperature is to measure performance at a range of temperatures within the lethal thermal limits. The resulting curve can be used to obtain a variety of scores including thermal optima (Huey and Kingsolver 1989). In amphibians, the rate of most physiological functions increases with temperature until function fails as the upper tolerance level is reached. However, thermal performance curves differ significantly between different physiological systems, making it unlikely that a single thermal optimum can exist for the whole animal. For example, locomotion is controlled by muscular, neural and metabolic activity, all of which have different temperature dependence (Rome et al. 1992).

2.1.1 Respiration

The complexity of respiration in many amphibians (including *Triturus*) reflects their dual nature as aquatic and terrestrial animals. Respiratory exchange surfaces include lungs, buccal cavity, skin and gills, each used to a greater or lesser extent depending on the species, stage of development and habitat. Water and air present different problems for respiration and most amphibians are adapted to dealing with both media. Since reproduction in *Triturus vulgaris* occurs only in water, I shall concentrate on the effect of temperature on bimodal respiration in aquatic amphibians.

The main differences between water and air as respiratory media are in density, viscosity, the rate of diffusion and the solubility of gases. The greater density and viscosity of water in relation to air means that more energy is needed to move water across the respiratory surfaces. The rate of diffusion of oxygen in water is about 10,000 times slower than in air (Schmidt-Nielson 1990) and the solubility of gases decreases with increasing temperature, dissolved oxygen concentration almost halving between 0 and 30°C (Table 2.1).

Temperature °C	O_2 Concentration (moles m^{-3})	
	Fresh water	Air
0	0.457	9.349
10	0.352	9.018
20	0.284	8.711
30	0.236	8.423
40	0.200	8.154

Table 2.1. The concentration of oxygen in water and air at different temperatures. Source: Denny (1993).

Not only temperature, but also turbidity and the time of day affect the oxygen content of water. For example, the balance between respiration and photosynthesis in a water body results in net oxygen production during the day but, at night, respiration alone produces a net loss of oxygen (Dejours 1976).

The most interesting aspects of bimodal respiration relate to the division of oxygen uptake between respiratory surfaces and the considerable variation that occurs in this partitioning under different environmental conditions (Rahn and Howell 1976). Since cutaneous respiration is predominantly limited by diffusion (Piiper 1988) the diffusion gradient between the environment and cellular fluid is of prime importance. Nevertheless, amphibians have evolved several adaptations to improve gaseous exchange via the skin including the lack of hair or scales, a thin epidermis, high vascularisation and (in anurans) the ability to increase blood flow to the skin capillaries during exercise. Frangioni and Borgioli (1989) suggest that newts have an additional mechanism, using the spleen to store blood in well-oxygenated environments and releasing this supply under conditions of hypoxia.

Although *Triturus vulgaris* has fully developed lungs, this species is able to spend considerable periods of time submerged, when it relies almost solely on cutaneous respiration. The buccal cavity is less well vascularised than the skin and is thought only to play a minor role (Czopek 1959). The visible ventilation of the buccal cavity is probably associated with the receipt of chemical cues (Halliday pers. comm.). Czopek (1959) studied the vascularisation of respiratory surfaces in breeding and non-breeding *Triturus vulgaris*. The length of capillaries

per gram body mass increased considerably in breeding individuals, mostly due to the development of the highly vascularised crest. In non-breeding individuals, the skin contained 74% and the lungs 24.5% of all respiratory capillaries. During the breeding season this was altered to skin 80%, lungs 19% in males and skin 76%, lungs 23% in females. Only the remaining 1% of respiratory capillaries were found in the buccal cavity. In addition, the epidermis of breeding newts was thinner and less horny. Czopek (1962) described an unpleasant experiment by Bannikov (1948) in which breeding and non-breeding *Triturus vulgaris* were kept in water without access to air to see how long they could survive. Breeding individuals of both sexes survived much longer (up to 100 hours for males and 80 hours for females) than non-breeding individuals (about eight hours only).

Oxygen uptake is affected by the availability of environmental oxygen and metabolic activity, both of which are influenced by temperature. The most common response of bimodal breathers to hypoxia (low partial pressure of oxygen in the environment), or to an oxygen debt following exercise, is to increase the proportion of oxygen acquired from the air (Pinder and Burggren 1986; Boutilier 1988). Specifically, this consists of an increase in respiratory rate rather than the volume of air taken in a single breath (Kruhøffer et al. 1987) because of the fixed volume of the buccal pump (Boutilier et al. 1992) (and because too much air taken in one breath prevents sinking, Halliday pers. comm). Halliday and Worsnop (1977) demonstrated that, in a constant temperature environment, the interval between breathing ascents was negatively correlated with activity in *Triturus vulgaris* but there was considerable variation between individuals.

In oxygen-rich environments and in the absence of strenuous activity, the response to a change in temperature is more variable. Some species maintain predominantly cutaneous respiration while others resort to pulmonary respiration with increasing temperature (Feder and Burggren 1985). Eddy and McDonald (1978) found that oxygen uptake in *Triturus cristatus* increases steadily with temperature from 5.7µl/min/10g at 5°C to 17µl/min/10g at 25°C. In

well-aerated water, the newts relied predominantly on cutaneous respiration, surfacing to breathe only about once every 8 minutes, even at 20°C. Under conditions of hypoxia, this rate increased to once every 3 – 4 minutes. They concluded that in well-aerated water, relatively inactive newts could remain submerged almost indefinitely, but with increasing levels of activity or decreasing levels in the partial pressure of oxygen, the oxygen requirement demanded supplementary pulmonary respiration. In 1962, Czopek repeated Bannikov's experiment but kept the water fully saturated with oxygen at 18 – 22°C. Czopek found that both breeding and non-breeding *Triturus vulgaris* survived without access to air for several months. There was no evidence that they would not have survived for longer had the experiment not been terminated (Czopek 1962).

The response to increased oxygen demand is further complicated if behavioural activities conflict with the ascent to the surface. Such a conflict may arise because of the overriding importance of the behaviour (flight from predators, prey capture, courtship) or because the action of surfacing itself incurs a cost (the threat of surface predators). Halliday and Sweatman (1976) carried out preliminary investigations into the conflict between breathing and courtship and found that, despite an increase in male activity, the inter-breathe interval was longer during sexual stimulation than in the absence of a female. However, Halliday (1977b) demonstrated that a reduction in oxygen supply resulted not only in a significant decline in spermatophore production, but also in a decrease in the duration of each sequence. The opposite effect, produced by an increase in oxygen supply, was much less marked. From observations of 117 breathing ascents during courtship, Halliday and Sweatman (1976) also showed that 89 occurred after the final sequence was completed, 12 at the end of an earlier sequence and 16 during retreat display. No ascent occurred during the crucial spermatophore transfer phase. It appears that males not only delay breathing during courtship but, under conditions of oxygen shortage, also decrease the duration of each sequence in order to complete as many depositions as possible.

2.1.2 Locomotion

Locomotor capacity in amphibians is greatly influenced by temperature, both in terms of oxygen demand and muscular activity. The onset of locomotion is accompanied by a rapid increase in the demand for oxygen, although in most cases the additional influx of oxygen to the cells is achieved only gradually. As oxygen consumption increases in response to demand, a steady state is reached. Steady state oxygen consumption in salamanders increases linearly with speed until maximum aerobic capacity is reached (Gatten et al. 1992).

Although the aerobic capacities of ectotherms are lower than those of birds and mammals, there is some evidence that a number of sustained but low-cost behavioural activities (for example, foraging and migration) can be supported largely by aerobic metabolism. At higher speeds of locomotion, aerobic metabolism cannot supply sufficient ATP for muscular activity. As a result, the use of anaerobic pathways increases and endurance decreases significantly. The degree to which each pathway contributes towards ATP production varies considerably between species (Bennett 1980; Gatten et al. 1992). Extremely rapid movement is almost entirely dependent on anaerobic respiration and results in early fatigue and a long recovery period. Within the amphibians, salamanders have lower resting metabolic rates and lower aerobic scope than anurans (Gatten et al. 1992).

Although there have been no direct measurements of the effect of temperature on the metabolic cost of sustained exercise in amphibians, its effect on nonsustainable activity has been more widely reported (Gatten et al. 1992). For example, a large increase in oxygen consumption (Q_{10} of about 2.0) was obtained between 10 and 15°C in *Taricha torosa* (Salamandridae) during forced activity in water (measured using a motorised rotation chamber) (Harlow 1978 in Gatten et al. 1992). The increase was met by aerobic metabolism. Anaerobic metabolism is less responsive to temperature with a Q_{10} of only 1.1 – 1.3 above 20°C (Bennett 1980). In this way, life-saving burst activity (such as flight from predators) is independent of temperature.

Many *in vitro* studies have demonstrated the temperature sensitivity of muscle properties. For example, maximum power production, maximum speed of shortening and twitch properties of muscle all have a large Q_{10} (3 – 4) at low temperatures. These properties affect different types of locomotion; cyclical movement (such as swimming) is related to twitch properties, since each muscle must activate and relax before the opposing muscle can be activated whereas sudden, isolated movement (such as a jumping) is dependent on the maximum speed of shortening (Else and Bennett 1987; Rome et al. 1992). In addition, the mechanical power of aerobic muscle fibres is reduced by one to two thirds at low temperatures, causing anaerobic fibres to be recruited at lower speeds and reducing the speed at which locomotion can be sustained (Rome et al. 1992).

During *in vitro* studies, the entire muscle is typically activated by electrical stimulation. *In vivo* studies, however, have revealed that during natural movement, not all muscle fibres are active at any one time. To some extent, the reduction in power at low temperatures can be alleviated by the recruitment of additional fibres by the nervous system. Maximum burst performance and maximum sustainable performance, however, remain temperature sensitive (Rome et al. 1992).

Although I have attempted to make a few generalisations concerning the relationships between temperature, respiration and locomotion, it must be emphasised that the majority of studies are based on relatively few species. This, together with considerable individual variation and the use of different methodologies, makes extrapolation to other species hazardous. In addition, the nature of locomotion in amphibians varies enormously. For example, the energetic demands for a frog, leaping in the air are likely to be very different to those of a newt displaying in water. The complex courtship display of *Triturus vulgaris* appears to contain both sustainable and nonsustainable elements and a mixture of cyclical movement (swimming and fanning) and rapid, isolated movement (move-to-the-front and whip). The extent to which aerobic and anaerobic respiration are used to meet oxygen demand

during courtship is not known for this species but, according to Pough et al. (1992), the vigorous courtship of these *Triturus* species is likely to be supported mainly by aerobic metabolism.

No previous work on the effect of temperature on courtship in smooth newts has been reported. The laboratory experiments described in this chapter were designed to examine whether temperature affected the successful transfer of spermatophores, in particular via its influence on locomotion (display rate) and the need for pulmonary respiration.

Experiment one: the effect of temperature on spermatophore transfer and other components of courtship.

Aim: ***to compare courtship in smooth newts at three different temperatures, corresponding to those experienced in the wild.***

Experimental hypotheses:

1. *Temperature affects the deposition and pick-up of spermatophores.*

2. *Temperature influences the probability of pick-up of particular spermatophores within an encounter.* This additional hypothesis was included to clarify some discrepancies between the results of research carried out by Halliday (1974; 1983) and Hosie (1992). Halliday found that the probability of pick-up by the female increased with successive spermatophores within a sexual encounter, and interpreted this as a means by which females choose to pick up from fitter males. Hosie, on the other hand, found that females were more likely to pick up the earlier spermatophores within an encounter. Halliday did not control the temperature, which ranged from 9 – 22°C, while Hosie's experiments were carried out in an unheated outdoor shed, where temperatures ranged from 5 – 20°C, but were mostly considerably colder than those of Halliday. It was thought possible that temperature differences could account for the inconsistencies between these two data sets.

3. *Temperature affects male display rate*. Teyssedre and Halliday (1986) showed that successful courtship was correlated with bouts of sustained display in which the interval between display acts was less than four seconds. If the locomotor ability of males is

depressed at low temperatures and enhanced at high temperatures, the interval between display acts would be expected to decrease with increasing temperature.

4. *Temperature affects the baseline receptivity of females.* The success of courtship depends not only on the effectiveness of the male's display, but also on the initial receptivity of females to that display (Halliday 1974; Hosie 1992). Within an experiment involving free courtship it is not possible to separate these factors, but it is feasible to ascertain if temperature influences the baseline receptivity of females by scoring female approaches towards the male (Hosie 1992).

2.2 Methods

2.2.1 Collection and maintenance of newts

Animals were obtained from Braystone Farm, Aspley Guise, where four new ponds were created by Philip Clarke in 1990. A great variety of aquatic and marginal plants were introduced and the ponds provide an excellent habitat for smooth and crested newts (*Triturus vulgaris* and *T. cristatus*), common frogs (*Rana temporaria*) and common toads (*Bufo bufo*).

A plastic drift fence was erected around one of the ponds at a distance of about 2m from the water's edge. The plastic was sunk to at least 5cm into the soil and supported by wooden stakes. Eight outer and three inner buckets were dug into the ground, adjacent to the plastic. In wet conditions, pieces of wood were provided in the buckets to help non- amphibious animals escape from water and in dry conditions, grass was used to prevent desiccation. The buckets were checked daily between 7am and 11am. All smooth newts were taken back to the laboratory for measurement before they were either kept for research or returned to the pond.

All females were collected from the drift fence (to ensure that they were unmated) whilst males were obtained both from the drift fence and from two adjacent ponds, using funnel traps. At the Open University field site, males and females were maintained in separate aquaria within large outdoor tubs containing water. This allowed the newts to experience natural light

and temperature fluctuations, while remaining buffered from extreme temperature changes. Newts were fed on *Daphnia* and tubifex *ad libitum*. All the animals, their eggs and larvae were returned to Braystone Farm as soon as possible after the experiments.

2.2.2 Experimental procedure

One observation and one settling tank were set up at three temperatures: high (18.5 – 20.5°C) using a thermostat aquarium heater; medium (13 – 14°C) at room temperature; low (6 – 8°C) by pumping water via an ice-making machine to cooling coils placed in the tanks. These temperatures lie within the normal range experienced by newts during the breeding season in Great Britain (Chapter three). Males and females were picked randomly from the stock aquaria and maintained at one of the three temperatures for 16 – 20 hours. This settling period was not intended to represent full metabolic acclimation (which takes 2 – 11 days in the wild (Rome et al. 1992)), but was implemented to avoid any behavioural anomalies arising from sudden transfer to a new temperature. Six pairs (two at each temperature) were observed during each session. Since the pair in the high temperature tank frequently courted within a few minutes, trials were begun 15 minutes earlier at this temperature in order to reduce the chances of sexual activity occurring simultaneously in all three tanks.

Each pair was left to settle in the observation tank for at least 15 minutes before the start of the trial, the sexes separated by a plastic partition. Once the partition was removed, courtship was observed and recorded on to a voice-activated tape-recorder. All aspects of sexual behaviour were noted but special attention was paid to whip and fan frequencies, fan bout duration and spermatophore transfer. Following at least one spermatophore deposition, trials were terminated when the newts had lost interest in one another (defined by a minimum of five minutes with no interaction). If no courtship occurred, the trial was stopped after one hour and the female was tested a second time at the same temperature, 48 hours later with a different male.

A total of 60 females was used (20 at each temperature). Temperature was recorded to the nearest 0.5°C at the beginning and end of each observation session. Slight variation could occur since the heating/cooling apparatus was removed for the duration of the session. The oxygen content of the water was measured after each trial using a portable dissolved oxygen meter. The number of dark/light hours were adjusted on a weekly basis to match London lighting-up times and varied from 12/12 hours to 15/9 hours. The experiment was carried out between 24 March and 29 April 1994.

2.3 Analysis and Results

2.3.1 Spermatophore transfer

Analysis was carried out on all courtships in which at least one spermatophore deposition occurred. Statistical tests are two-tailed unless otherwise indicated. The total number of depositions and pick-ups at each temperature were calculated (Table 2.2 and Figure 2.1).

Temperature	Low	Medium	High
Courtships	16	18	18
Deposition	47	64	63
Pick-up	32	43	29
% Pick-up	68	67	46

Table 2.2. Deposition and pick-up at the three temperatures.

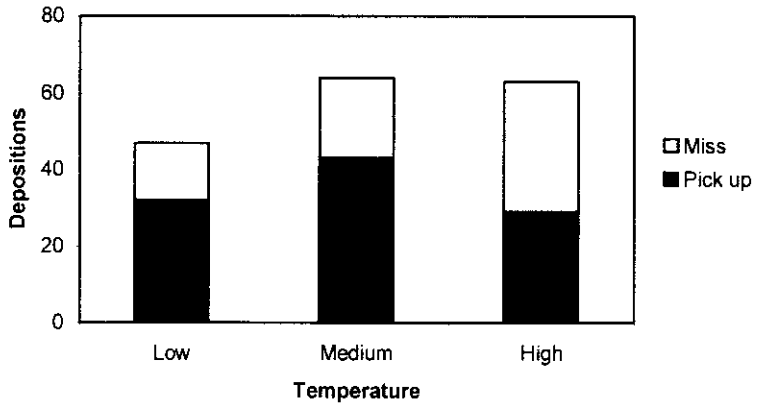


Figure 2.1. Deposition and pick-up at the three temperatures.

These totals were obtained from an equal set of opportunities for spermatophore transfer at each temperature (20 females, with a maximum of two trials each) and can therefore be used

directly in statistical analysis. Chi-squared analysis revealed a significant difference between the proportions deposited and picked up at the three temperatures (Statistics box 2.1A). The number of spermatophores deposited at high and medium temperatures (63 and 64) was considerably higher than at the low temperature (47). However, since the percentage pick-up was greater at low and medium (68% and 67%) than at the high temperature (46%), spermatophore transfer was most successful at the medium temperature. The most logical way to partition the 3×2 chi-squared contingency table was to separate the low and medium from the high temperature (Table 2.2). There was no significant difference between low and medium, but a highly significant difference between low + medium and high (Statistics box 2.1A) indicating that the statistically significant change in proportions between pick-up and miss occurred between the medium and high temperatures.

Table 2.3 gives details of pick-up in successive sequences within single encounters.

Statistics box 2.1

A. Chi-squared test to compare the number of spermatophores picked up and missed at the three temperatures (3×2 contingency table). $\chi^2 = 6.54$, df = 2, $\mathbf{p < 0.05}$. 1st partition, low and medium: $\chi^2 = 0.09$, df = 1, $p > 0.7$. 2nd partition, low + medium and high: $\chi^2 = 7.75$, df = 1, $\mathbf{p < 0.01}$.

B. Chi-squared test to compare pick-up of the first three sequences within each temperature (3×2 contingency tables). Low: $\chi^2 = 1.57$, df = 2, $p > 0.5$. Medium: $\chi^2 = 2.28$, df = 2, $p > 0.2$. High: $\chi^2 = 0.86$, df = 2, $p > 0.5$.

D. Chi-squared test to ascertain whether spermatophore pick-up was influenced by the outcome of the previous sequence. Low: $\chi^2 = 0.27$, df = 1, $p > 0.5$. Medium: $\chi^2 = 0.04$, df = 1, $p > 0.5$. High: $\chi^2 = 0.44$, df = 1, $p > 0.5$.

Temperature		Low	Medium	High
Sequence one	**Deposition**	16	18	18
	Pick-up	10	11	10
	% Pick-up	62.5	61.1	55.5
Sequence two	**Deposition**	14	18	17
	Pick-up	11	15	9
	% Pick-up	78.6	83.3	52.9
Sequence three	**Deposition**	12	18	13
	Pick-up	7	12	5
	% Pick-up	58.3	66.6	38.5
Sequence four	**Deposition**	5	9	9
	Pick-up	4	4	4
	% Pick-up	80	44.4	44.4

Table 2.3. Deposition and pick-up from sequences one to four within an encounter.

There was no significant difference in the probability of pick-up in sequences one, two and three within each of the three temperatures (chi-squared test, Statistics box 2.1B). Sequence four was not included in statistical analysis because the sample size was too small. A difference in temperature, therefore, does not explain the discrepancies between results from previous experiments (Halliday 1983; Hosie 1992) and the present data support neither an increase nor a decrease in the probability of pick-up with successive sequences.

Chi-squared tests were also carried out in order to ascertain whether spermatophore pick-up was influenced by the outcome of the previous sequence. Expected values were calculated from the actual results assuming independence of successive spermatophores. Again, none of the differences was significant, indicating that the probability of pick-up is not affected by the success or failure of the previous attempt at spermatophore transfer (Statistics box 2.1D).

2.3.2 Male display rate, female receptivity and the speed of spermatophore transfer

Why were deposition rates higher at medium and high temperatures? Two factors likely to affect deposition rate are the effectiveness of the male's display and the baseline receptivity of the female.

The effect of temperature on male *display rate* was investigated by calculating the frequency distribution of the interval between display acts (whip – whip, whip – fan, fan – whip) and the duration of fan bouts. Whips are examples of behavioural events (patterns of short duration) whereas fanning, which can last a considerable time, is more analogous to a behavioural state (Martin and Bateson 1993). Whip – whip, whip – fan and fan – whip were therefore combined to give a composite score, describing the speed at which males moved from one display act to the next. The duration of fan bouts was treated separately. Courtships were first standardised by discarding all display following the final pick-up (termed residual display by Halliday 1976) and unsuccessful display during the first attempts at orientation (following which the female turned away for one minute or more).

The problem with analysing interval data is deciding when one act follows another legitimately within the same bout of courtship, or when the interval is long enough to be described as a pause. The bout criterion interval (BCI) can be established using log survivorship analysis (Teyssedre and Halliday 1986; Martin and Bateson 1993) and differentiates between intervals that can be categorised as 'within-bout' and those which are 'between-bout'. If there is a clear difference between these intervals the analysis will produce a curve with two distinct parts, the first steep part of the curve representing within-bout intervals and the second shallow part representing between-bout intervals. This type of curve was not obtained from my results and I therefore concluded that no distinction between within- and between-bout intervals could be made. Nevertheless, it is still interesting to look at the difference in the distribution of display intervals between the three temperatures (Figure 2.2). In order to illustrate the complete frequency distribution, information for all individuals was pooled (16, 18 and 18 males at low, medium and high temperatures respectively).

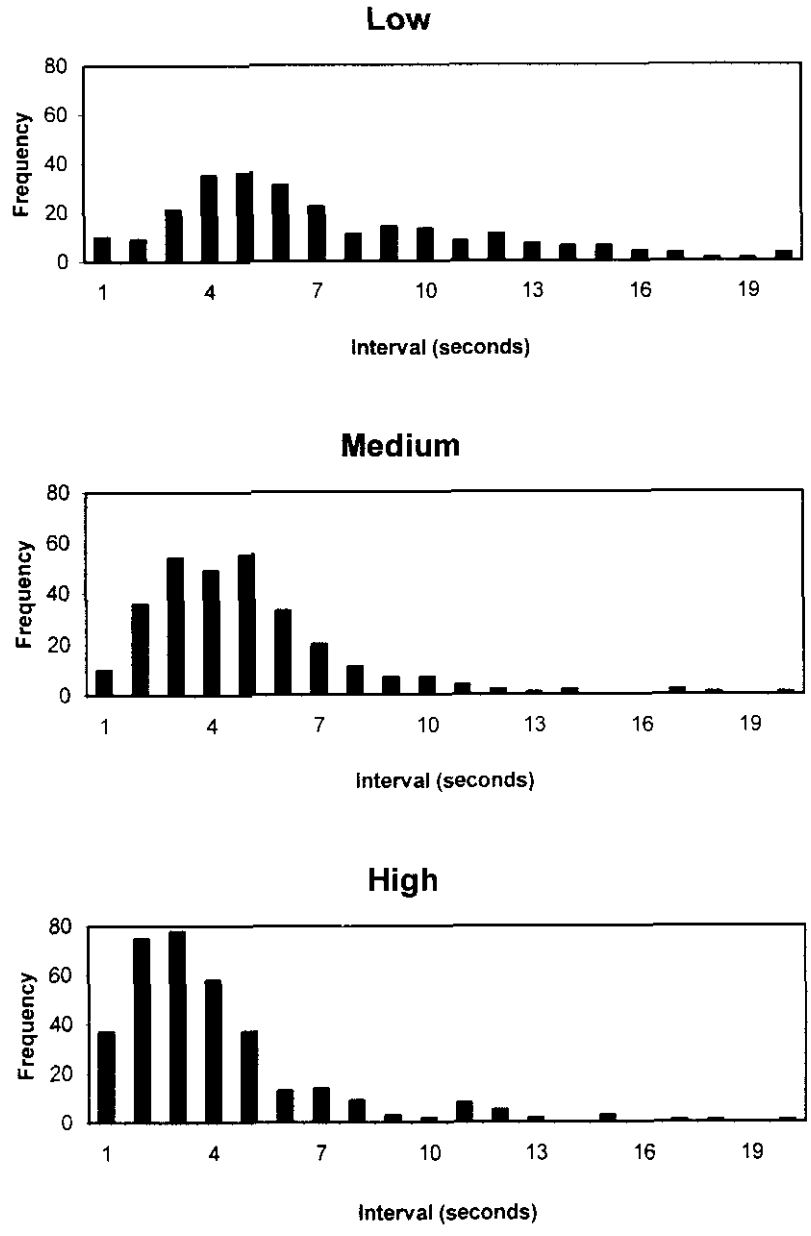


Figure 2.2. Frequency distribution of the intervals between whip – whip, whip – fan and fan – whip at three temperatures.

Both the display rate and the number of display events increased with increasing temperature. The median score from each individual (with a sample size of at least five intervals) was used for the purpose of statistical analysis. Figure 2.3 shows the relative cumulative distribution of the median interval between display events.

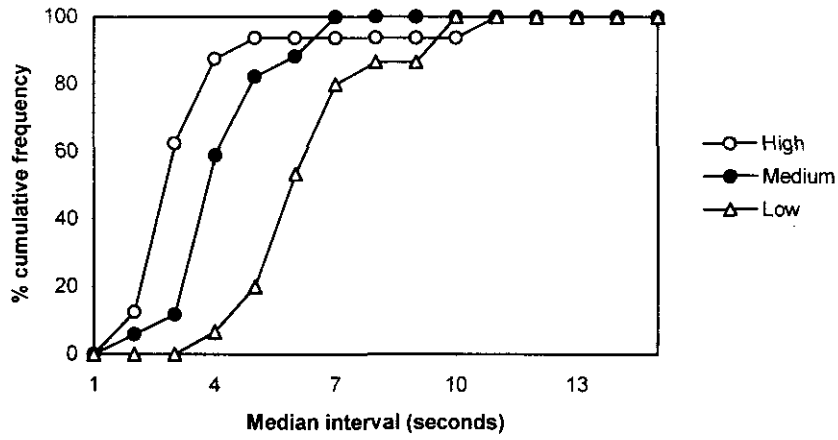


Figure 2.3. Percentage cumulative distribution of intervals between display events (median scores).

Gridlines are included to illustrate the percentage of display which occurred within an interval of four seconds or less, since this timing was identified by Teyssedre and Halliday (1986) as being critical to successful courtship. The percentage was 87.5, 58.8 and 6.6% at high, medium and low temperatures respectively.

The Kolmogorov-Smirnov test is designed to test differences between relative frequency distributions but is only available for comparison between two data sets. Since the direction of change in interval was predicted to increase from low to high temperatures, I carried out two one-tailed tests: a) between low and medium and b) between medium and high temperatures and reduced the alpha level to 0.01 (Bonferroni's adjustment). The difference between low and medium was significant at $\mathbf{p < 0.01}$ while that between medium and high just failed to reach a probability of 0.01 but was significant at $\mathbf{p < 0.05}$ (Statistics box 2.2).

Statistics box 2.2.

Kolmogorov-Smirnov test to compare the cumulative relative frequency distribution of intervals between display events (median scores taken for each individual).

Low and medium temperatures: $D_{mn} = 158$. Critical value ($\alpha 0.01$) = 130. $\mathbf{p < 0.01}$.

Medium and high temperatures: $D_{mn} = 138$. Critical value ($\alpha 0.01$) = 139. $\mathbf{p < 0.05}$.

The duration of fanning bouts is illustrated in Figure 2.4. Although there were insufficient data to obtain a median score for each individual and carry out statistical tests, the graphs show that fanning bouts tended to be longer and more numerous at the low temperature.

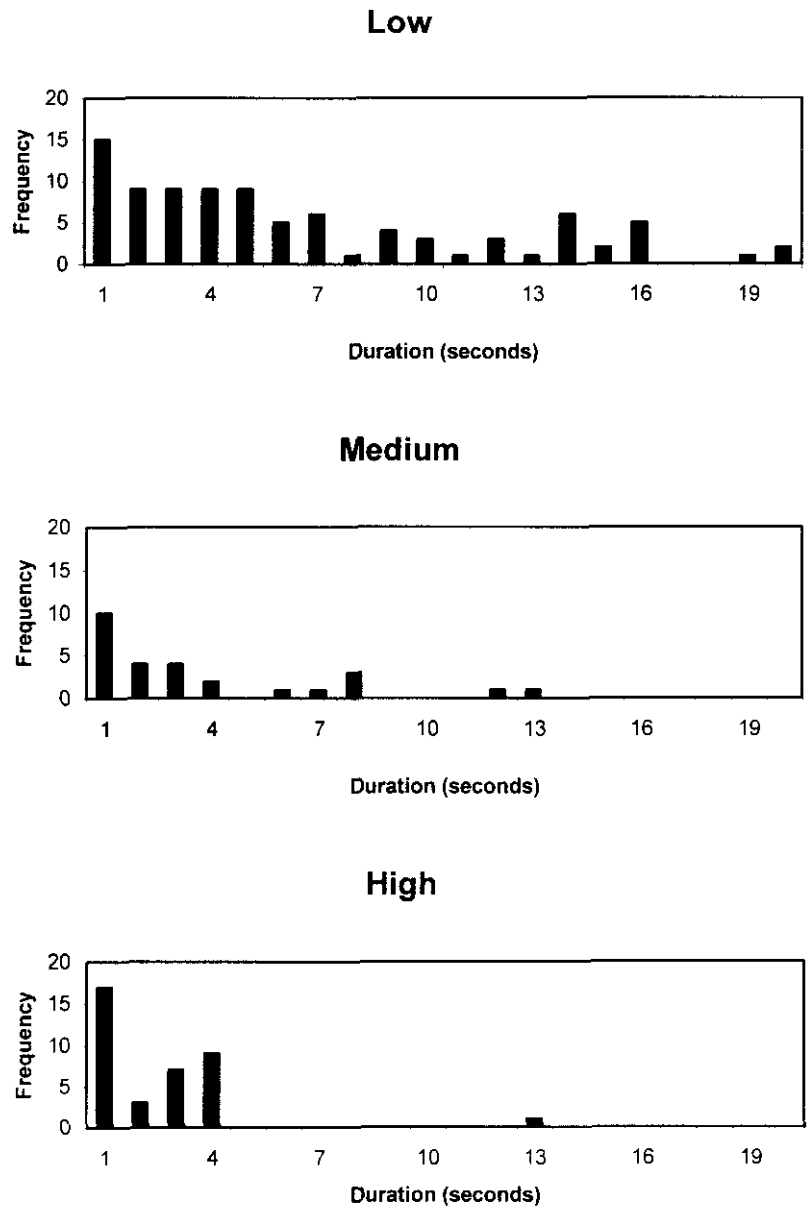


Figure 2.4. Frequency distribution of the duration of fanning bouts.

Female *baseline receptivity* may also have affected deposition rate at the three temperatures. Hosie (1992) divided female courtship behaviour into two parts; phase 1 involved behaviour directed towards the male before he began to display, and phase 2 consisted of behaviour which was in response to male courtship. Phase 1 activities are thus performed by females with a high baseline receptivity. I used two activities (defined by Hosie

1992) to score courtships with phase 1 behaviour:- 'distant approach' in which the female approached the male to a distance of greater than one body length and 'close approach' in which the female approached the male to a distance of less than one body length.

In the smooth newt, reproductive state is an important influence on female sexual motivation, unmated females being more proceptive than their mated counterparts (Hosie 1992). Since these courtships involved unmated females only, any additional effect of temperature on motivation could be ascertained (Table 2.4).

Courtships	Low	Medium	High
With phase 1	1	6	8
Total	15	17	18

Table 2.4. The number of courtships which began with phase 1 behaviour by females at the three temperatures.

Although there was an increase in the number of females demonstrating phase 1 behaviour at the medium and high temperatures, this was not significant (chi-square: $\chi^2 = 2.725$, df = 2, $p > 0.2$).

Why was pick-up more successful at medium and low temperatures? Failure to pick up may be attributed to choice by the female or to accident (such as the incorrect positioning of the spermatophore). During this experiment, I observed many females with spermatophores stuck to their legs, stomachs and chins, and on several occasions spermatophores remained attached to the male's cloaca. Although this experiment was not designed to investigate female choice, I was able to examine the potential for accidental failure to pick up by comparing the speed of display during the spermatophore transfer phase. I used the number of seconds between creep and deposition to represent the speed of spermatophore transfer since these are two events for which it was possible to obtain accurate times. A single factor ANOVA,

followed by Tukey tests on the time between creep and deposition during the first sequence of each courtship, revealed a significant difference between low and medium temperatures but no difference between medium and high temperatures (Statistics box 2.3 and Figure 2.5).

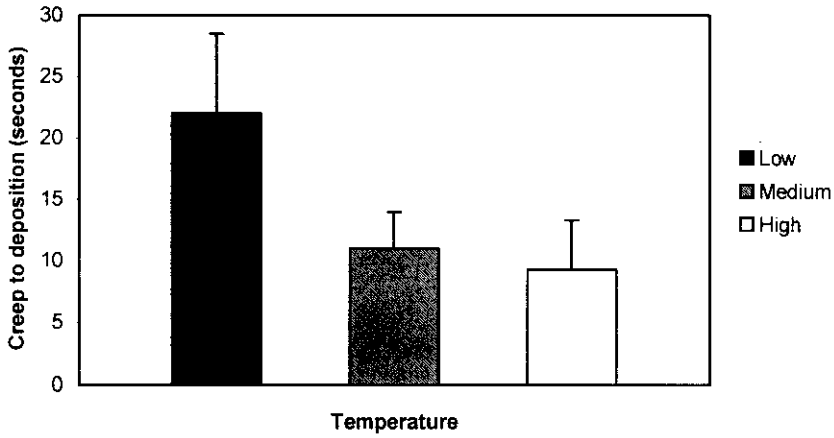


Figure 2.5. The number of seconds between creep and deposition (mean + SD).

The difference in time taken from creep to deposition at medium and high temperatures was thus too small to explain the difference in pick-up success.

Statistics box 2.3.

Single factor ANOVA to compare the number of seconds between creep and deposition at low, medium and high temperatures. $F = 32.7$, $df = 2$, $\mathbf{p < 0.001}$. The Tukey test established a significant difference between low and both high and medium temperatures but no difference between medium and high temperatures ($\mu_{low} \neq \mu_{medium} = \mu_{high}$).

Low vs. high: difference between means = 13.17, n = 13, SE = 1.21, q = 10.88, q(0.001,40,3) critical value = 5.528, $\mathbf{p < 0.001}$.

Low vs. medium: difference between means = 11.46, n = 14, SE = 1.26, q = 9.09, q(0.001,40,3) critical value = 5.528, $\mathbf{p < 0.001}$.

Medium vs. high: difference between means = 1.71, n = 17, SE = 1.19, q = 1.43, q(0.2,40,3) critical value = 2.47, $p > 0.2$.

2.3.3 Summary of results from experiment one

1. Depositions were more frequent at medium and high temperatures but percentage pick-up was higher at medium and low temperatures. Spermatophore transfer was therefore most successful at the medium temperature. *Reject null hypothesis one that temperature has no effect on the deposition and pick-up of spermatophores.*

2. There was no difference in pick-up success of successive spermatophores within an encounter. *Accept null hypothesis two that temperature has no effect on the probability of pick-up of particular spermatophores within an encounter.*

3. The interval between display events decreased with increasing temperature. *Reject null hypothesis three that temperature has no effect on male display rate.* Fanning bouts appeared to be more numerous and longer at the low temperature but this could not be tested statistically.

4. Female initiation of courtship was marginally reduced at the low temperature, but this was not statistically significant. *Accept null hypothesis four that temperature has no effect on female baseline receptivity.*

5. The difference in pick-up success between medium and high temperatures could not be attributed to the speed at which the male moved from creep to deposition during the spermatophore transfer phase.

In this experiment newts were allowed to continue courtship after ascending to the surface for air. Under more natural conditions courtship may be terminated by an ascent to the surface. The next experiment asks how temperature affects the need to breathe air and whether this in turn influences spermatophore deposition.

Experiment two: the effect of temperature on the need to breathe air.

Aim: ***to compare the interval between breathing ascents during male smooth newt courtship at three different temperatures.***

Experimental hypotheses:

1. *Temperature affects the time taken between breaths.* Halliday (1977b) created conditions of reduced, normal and excess oxygen availability using nitrogen, compressed air and oxygen bubbled through the experimental tanks. He found a positive association between the length of the first inter-breathe interval, the length of individual sequences and oxygen supply. In addition, significantly fewer spermatophores were produced under nitrogen than under air during the first inter-breathe interval. The same effects are expected as a result of temperature-induced differences in oxygen availability.

2. *High-crested males are able to remain submerged for longer during courtship.* Bannikov and Czopek (1962) demonstrated that the crest was essential for survival in water without access to air, unless the water was kept fully aerated. Czopek (1962) also showed that, although the crest of breeding individuals became reduced a few days after submersion, non-breeding individuals eventually developed some form of crest. I predicted that the increased respiratory surface provided by larger crests would enable males to remain submerged for longer during vigorous activity.

2.4 Methods

Tail-depth varies in proportion to crest height (Baker 1992b) and being a more reliable measurement, was used in preference. Tail-depth was determined for a stock of 30 males from which two groups of males were chosen: 'high-crested' (13 – 15mm) and 'low-crested' (8 – 10mm) while ensuring a range of body sizes in both groups. Since these males were captured with funnel traps, their mating history was unknown. They were kept apart from females for at least two days before testing to ensure a full spermatophore supply (Verrell 1986b). Testing took place at two temperatures: high (19.0 – 20.5°C) and low (7.0 – 9.0°C) and the males were

allowed to settle at the appropriate temperature for 16 – 20 hours. Females were anaesthetised using MS222 and placed in a strait-jacket (Halliday 1975) allowing complete control of female responses during courtship. All females were between 46 and 48mm snout vent length, and were anaesthetised on no more than two occasions.

The experimental procedure followed a similar design to 'experiment 4' in Halliday and Sweatman (1976). Each male was allowed to settle in the observation tank for 15 minutes. As soon as the male breathed following this period, the female was introduced and recording began. After a static period of two minutes, during which the female was held stationary in front of the male, she was manipulated to give a positive response to every part of male courtship, thus encouraging the male to reach deposition in as many successive sequences as possible. The trial ended when the male breathed for a second time or after a maximum of 75 minutes. Details of male display were recorded on to a hand-held tape- recorder. Females were removed if no display occurred within the first three minutes.

In 1994, seven animals (four high-crested and three low-crested) were tested at both temperatures, with the order of test temperature reversed for successive individuals. The second trial was carried out 48 hours later to allow for sperm replenishment (Verrell 1986b). Twelve additional males of mixed crest height were tested at the high temperature.

In 1995, the experiment was repeated with 11 males at an intermediate temperature (12.5 – 13.5°C).

2.5 Analysis and Results

2.5.1 Inter-breathe interval

The time taken for each male to return to the surface to breathe (inter-breathe interval) was calculated and plotted against temperature (Figure 2.6).

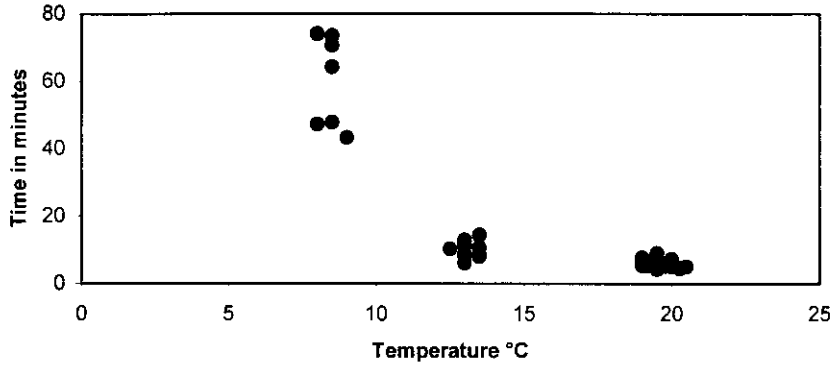


Figure 2.6. Temperature and inter-breathe interval.

Figure 2.6 represents data from all individuals including the seven which were tested twice (at both low and high temperatures). For the purposes of analysis, the high temperature data from these individuals were omitted so that all samples contained independent subjects and the three temperatures could be analysed simultaneously. There was a significant difference in inter-breathe interval between the three temperatures (Statistics box 2.4).

The most important factors affecting the need to surface for air during courtship are energy consumption (related to display rate) and the dissolved oxygen content of the water. Display rate was calculated using interval data in the manner described in section 2.3.2 above. The median score (whip – whip + whip – fan + fan – whip) was calculated for each male and

Statistics box 2.4.

A. Kruskal-Wallis test to compare inter-breathe interval at low, medium and high temperatures. $H = 21.55$, $df = 2$, $\mathbf{p < 0.001}$. Multiple comparisons using mean ranks (Zar 1984 p200). Low and high: $q = 4.6$, $\mathbf{p < 0.001}$. Low and medium: $q = 2.46$, $\mathbf{p < 0.05}$. Medium and high: $q = 2.41$, $\mathbf{p < 0.05}$.

B. Kolmogorov-Smirnov test to compare the cumulative relative frequency distribution of intervals between display events. Low and medium temperatures: $D_{mn} = 77$, critical value (α 0.01) = 53, $\mathbf{p < 0.01}$. Medium and high temperatures: $D_{mn} = 50.16$, critical value (α 0.1) = 54, $p > 0.1$.

expressed as % cumulative frequency (Figure 2.7). The duration of fan bouts was ignored since this is frequently determined by the female (Halliday 1975).

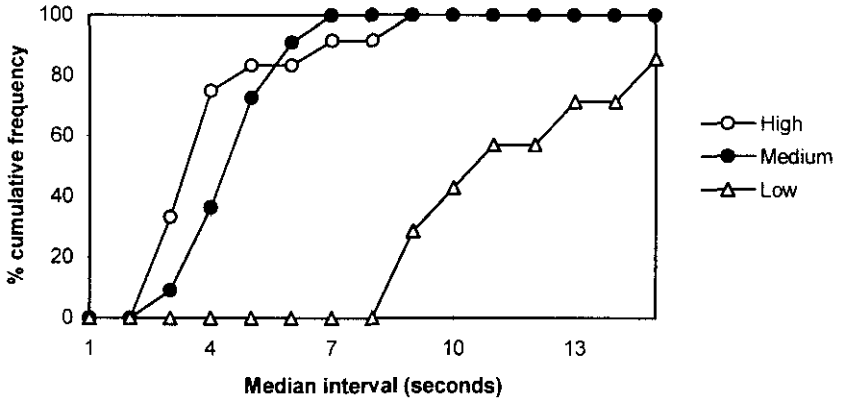


Figure 2.7. Percentage cumulative frequency distribution of the intervals between display events (whip – whip + whip – fan + fan – whip).

The overall pattern was similar to that obtained for free courtship in experiment one (see Figure 2.3) although the difference between display rate at medium and high temperatures was no longer significant (Kolmogorov-Smirnov $p > 0.1$, Statistics box 2.4B). At the low temperature there was an even greater skew towards long intervals between display events, reflecting the reluctance of the males to continue courting until they needed to breathe. The differences in inter-breathe interval thus coincided with a slight increase in display rate between medium and high temperatures, and a considerable increase between low and medium temperatures.

Having established that males surface to breathe earlier at higher temperatures during courtship, it is necessary to determine whether this has an effect on spermatophore transfer. Since an anaesthetised female was used for this experiment, only data for spermatophore deposition are available, but these can be compared with results from the previous experiment (Table 2.5).

Number of depositions per encounter		1	2	3	4	5	6	7	Median
Experiment one	**High**	1	4	4	6	1	1	1	3.5
	Medium	0	0	9	8	1	0	0	3.5
	Low	2	2	7	5	0	0	0	3
Experiment two	**High**	1	4	7	0	0	0	0	3
	Medium	1	3	5	2	0	0	0	3
	Low	0	0	3	2	1	0	1	4

Table 2.5. The frequency distribution of spermatophores per encounter at high, medium and low temperatures: a comparison between experiments one and two.

In contrast to experiment one, there were few encounters containing more than three spermatophores at high and medium temperatures and the frequency distributions of spermatophores per encounter were similar. At the low temperature, the number of spermatophores per encounter increased as males were encouraged to court for artificially long periods.

Although the dissolved oxygen meter was not always reliable, the line produced by the best readings compared favourably with the figures for fresh water given in standard textbooks (Figure 2.8).

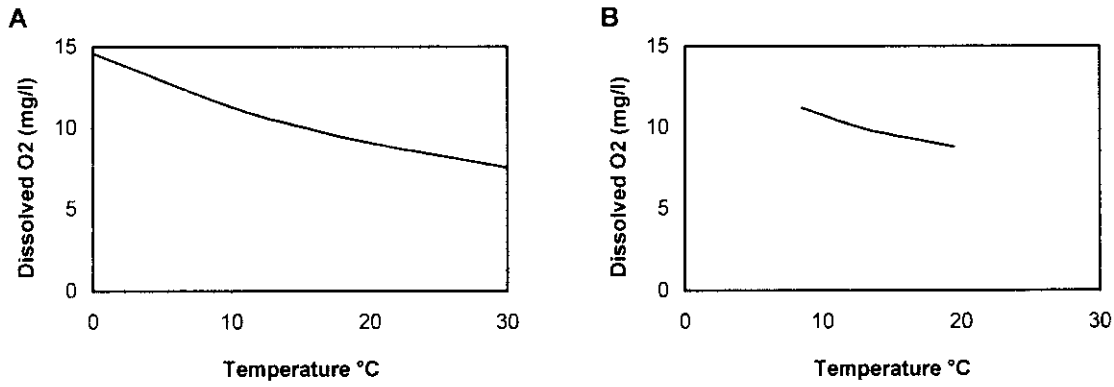


Figure 2.8. A: standard readings for oxygen solubility in pure water. Source: Table IVB of 'International Oceanographic Tables', Volume 2, National Institute of Oceanography of Great Britain and UNESCO, 1973 in the manual for the dissolved oxygen meter. **B: readings obtained during the experiment.**

The relationship between temperature and dissolved oxygen was approximately linear over the temperature range in question.

2.5.2 Crest height

There was no difference in inter-breathe interval between high- and low-crested males at either the high or low temperatures (Statistics box 2.5).

2.5.3 Summary of results from experiment two

	Mean inter-breatheinterval. Minutes (seconds)	Median interval between display events (seconds)	Median number of depositions	Range in number of depositions	Oxygen availability (mg/l)
High	5.85 (351)	4	3	1 – 3	8.8
Med.	9 (540)	5	3	1 – 4	9.9
Low	60.2 (3601)	12	4	3 – 7	11.2

Table 2.6. Summary of results from experiment two.

1. At the low temperature, the length of the inter-breathe interval eliminated oxygen as a limiting factor in smooth newt courtship. At medium and high temperatures, the mean inter-breathe intervals were seven- and ten-fold shorter, a result of both the decrease in oxygen availability and the increase in display rate (Table 2.6). *Reject null hypothesis one that temperature does not affect the time taken between breaths.*

2. There was little difference in median number of spermatophores per encounter between temperatures, although the range was greater at the low temperature (Table 2.6).

3. Crest height had no effect on the ability to sustain courtship under water. *Accept null hypothesis two that there is no difference between the ability of high and low-crested males to remain submerged during courtship.*

Statistics box 2.5.

Robust rank order test to compare inter-breathe interval at low temperature between low- and high-crested males. This test was chosen due to the very small sample size (3 low-crested and 4 high-crested males) (Siegel and Castellan 1988). m = 3, n = 4, $\grave{U}$= 0.59, p > 0.2.

Mann-Whitney U test to compare inter-breathe interval at high temperature between low- and high-crested males. m = 7, n = 9, U = 31, p > 0.6.

2.6 Discussion

2.6.1 Temperature and courtship

In the first experiment a greater number of depositions were recorded at the high and medium temperatures, whereas pick-up was more successful at the medium and low temperatures. The optimal thermal range for each part of spermatophore transfer therefore overlapped at the medium temperature (13 – 14°C). High deposition rates were accompanied by an increase in display rate. Teyssedre and Halliday (1986) demonstrated that successful courtship is associated with longer bouts of display in which the interval between display events is four seconds or less. In this experiment, high deposition rates coincided with courtship in which at least 59% of display intervals were less than or equal to four seconds. At the low temperature only 6.6% of display intervals reached this criterion.

The high deposition rates at medium and high temperatures also coincided with a slight increase in female baseline receptivity (measured by the presence of phase 1 behaviour) and a decrease in the number and duration of fanning bouts. At the low temperature females appear to require a greater degree of stimulation by fanning to reach retreat display and spermatophore transfer.

Unlike deposition, pick-up was less successful at the high temperature. It was suggested that accidental failure to pick up may be caused by the rapid spermatophore transfer at the high temperature. However, there was no significant difference in the number of seconds between creep and deposition at the medium and high temperatures. It is possible that the time taken for the male to move from deposition to brake is more crucial in determining the correct positioning of the female, but I had no accurate measurements for this. An alternative hypothesis, that females pick up or miss spermatophores through choice has some supporting evidence. Female choice for higher-crested males has been demonstrated in the laboratory (Chapter four) and according to Green (1991a) this preference is exercised at the spermatophore pick-up stage. Furthermore, Waights (1996) demonstrated that the proportion

of pick-ups is significantly higher during encounters in which females interfere with other courtships (Chapter four), implying that highly motivated females pick up more spermatophores.

The constraints of experimental design permitted me to obtain data for three temperatures only, and did not allow the construction of a complete thermal performance curve. What is immediately striking, however, is the non-linearity of the relationships between temperature and the measures of courtship behaviour (Table 2.7).

	Low	Medium	High
Depositions	47	64	63
% pick-up	68	67	46
Examples of phase 1 behaviour	1	6	8
% display intervals of 4 seconds or less	6.6	59	87
Time to onset of courtship (minutes)	11.5	5.5	4.5
Creep to deposition (seconds)	21	11	8

Table 2.7. The non-linear nature of the relationship between courtship and temperature.

For all scores except pick-up, results from the medium temperature were closer to those from the high than the low temperature. The most significant physiological changes thus appear to take place between 8 and 13°C (typical pond temperatures during spring) and the effect is much less pronounced between 13 and 18°C. This may reflect the decrease in Q_{10} as temperature rises. It is essential for future work to investigate the changes which take place between 8 and 13°C in more detail.

Temperature did not affect the order of pick-up within an encounter and pick-up of a spermatophore was not influenced by the outcome of the previous attempt. There is thus no evidence from this or other (Verrell 1982a; Green 1991a) experiments to support a preference by female newts for the first or last spermatophores within an encounter. Indeed, it is not even clear whether the female is aware of the success or failure of pick-up. The importance of such preferences is probably diminished in the wild where sequences containing more than one or two depositions are rare (Chapter three).

2.6.2 Temperature and the need to breathe air

Results from the second experiment showed that temperature has a significant effect on the first inter-breathe interval during courtship. The ability to remain under water during courtship depends on energy consumption (display rate) and oxygen availability. The relationship between display rate and temperature was very similar to that found during free courtship, although the difference between medium and high temperatures was less marked. Surprisingly, this experiment, which was designed to elicit maximum display from males by presenting a 'highly responsive' female, resulted in display that was marginally slower than during free courtship (compare Figure 2.2 and Figure 2.4). Evidently, even a motivated female in a strait-jacket is not quite as good as the real thing! Since the use of a strait-jacket allowed me to test males with 'equally motivated' females, the differences in display rate could be attributed to the effect of temperature. This adds support to the relationship between display rate and temperature recorded in the first experiment in which the activity of the female was not controlled.

The availability of oxygen within a pond is variable and is dependent on a complex interaction between factors (Chapter three), but within the laboratory aquaria (as with samples of clean water) the relationship between dissolved oxygen and temperature was approximately linear (Figure 2.8). On the other hand, both inter-breathe interval and display rate varied in a non-linear fashion with temperature; between 13 and 18°C there was a difference of 1.1mg/l in dissolved oxygen content, one second in the median display interval and three minutes of courtship; between 8 and 13°C there was a similar difference of 1.3 mg/l in dissolved oxygen but seven seconds in the median display interval and 50 minutes of courtship. Once again, more work is needed to understand the physiological changes which occur between 8 and 13°C, but it is clear that smooth newt courtship can be sustained by cutaneous respiration alone at temperatures of 8°C or below. Spurway and Haldane (1953) obtained inter-breathe intervals for *Triturus cristatus* of 32.4 minutes at 10°C and 5 – 10 minutes at 20°C, both of

which correspond well with the data from experiment two. Halliday's (1977b) experiment, in which the dissolved oxygen content of water was manipulated using nitrogen, compressed air and pure oxygen, was carried out at room temperature. He obtained a relatively small difference in mean inter-breathe between the three levels of oxygen; 3.5 minutes, 5 minutes and 8 minutes resulting from reduced, normal and excess dissolved oxygen respectively. An increase in oxygen levels appears only to allow a predominance of cutaneous respiration when combined with a low temperature (and low display rate). The highly vascularised skin of breeding individuals which is important in survival under water (Czopek 1959) is thus not able to support the vigorous courtship activity at higher temperatures. Moreover, the additional respiratory surface provided by higher crests is not sufficient to increase the time spent submerged during courtship, at any temperature.

The energetics of courtship has been examined in only a few species of salamander. Bennett and Houck (1983) estimated oxygen consumption to increase by only 38% above resting levels during courtship in *Desmognathus ochrophaeus* (a small, lungless, terrestrial salamander) at 15°C, 90% of which was sustained aerobically. However courtship in this species, although lengthy, involves no vigorous display. Verrell (1985a) found a negative correlation between the rate of display and the duration of amplexus in the aquatic *Notophthalmus viridescens*. However, during amplexus, there are periods of rest between display bouts and males are able to remain under water for as long as three hours at 20°C. In contrast, *Ambystoma maculatum* continue to gulp air during their very vigorous courtship (Bennett and Licht 1974). Green (1991b) demonstrated a trade-off between the frequency and duration of fanning in the courtship *T. cristatus*. As courtship progressed fanning became slower, the length of courtship bouts decreased, and the interval between them increased, although these effects were only significant in the presence of a rival male (Chapters four and five). Despite this evidence of gradual fatigue during courtship, few encounters were terminated by the need to breathe. These experiments were carried out at 10 and 15°C, a

temperature span over which the decrease in oxygen availability may become significant. Unfortunately, the results were pooled and the effect of temperature is not known.

In the present study, it was evident that males were reluctant to surface at both medium and high temperatures. Breathing air only conflicts with courtship, however, if two conditions are met: first that the encounter is interrupted permanently by the ascent and second that the success of spermatophore transfer is reduced. During experiment one, males were able to resume courtship after breathing, but this is not surprising within the confined space of an aquarium and in the absence of rival males. The extent to which courtship in the wild is disrupted by an ascent to breathe is not known.

The effect on spermatophore transfer can be assessed by comparing the frequency distribution of depositions per encounter between experiments one and two (Table 2.5). There was a reduction in spermatophore deposition at the medium and high temperatures when courtship was terminated after a single ascent to the surface. However there is evidence to suggest that, in a more natural situation, males rarely deposit more than two or three spermatophores per encounter (Chapters three and four). Even in the shortened time available for courtship at medium and high temperatures, the rapid display was enough to ensure deposition of a reasonable complement of spermatophores. Indeed, Halliday (1977b) suggested that the compression in sequence length he observed under conditions of reduced oxygen occurred *in order* to compensate for the decrease in time available before the need to surface for air.

In the introduction, I discussed how the thermal performances of different physiological systems can vary. These experiments have shown that, in the laboratory, different parts of smooth newt courtship function optimally at different temperatures: for example, deposition (above 13°C), pick-up (below 18°C) and the ability to continue display without breathing (below 13°C). In addition to thermal performance, thermal preference (the body temperature

maintained by an ectotherm in a temperature gradient) is frequently investigated in the laboratory. Strübing (1954) estimated the preferred temperature for adult terrestrial *Triturus vulgaris* to be between 20.3°C (spring captures) and 23.5°C (captive bred) which according to the results presented here, is not compatible with the optimal temperature for courtship. However Katz et al. (1997) demonstrated in the laboratory that several anurans species (with varying geographical distributions) share a narrow range of unusually high preferred temperatures. The authors proposed that the preferred temperature represented a safe upper limit which enabled anurans to avoid high lethal temperatures.

2.6.3 Conclusions

Courtship at low temperatures was characterised by a low rate of deposition, a slow rate of display and (to some extent) a reduction in female motivation. On the other hand, pick-up success was high and courtship could be maintained for long periods by cutaneous respiration alone. Courtship at high temperatures was characterised by a high rate of deposition, rapid display and a slight increase in female motivation. However, pick-up success was poor and the need to surface for air reduced the time available for courtship to a few minutes. Despite the fact that there were not sufficient data to obtain a true thermal performance curve, I suggest that, in physiological terms, the optimal temperature for smooth newt courtship lies just below 13°C where spermatophore transfer success is high, but oxygen availability has not yet become a limiting factor (Figure 2.9).

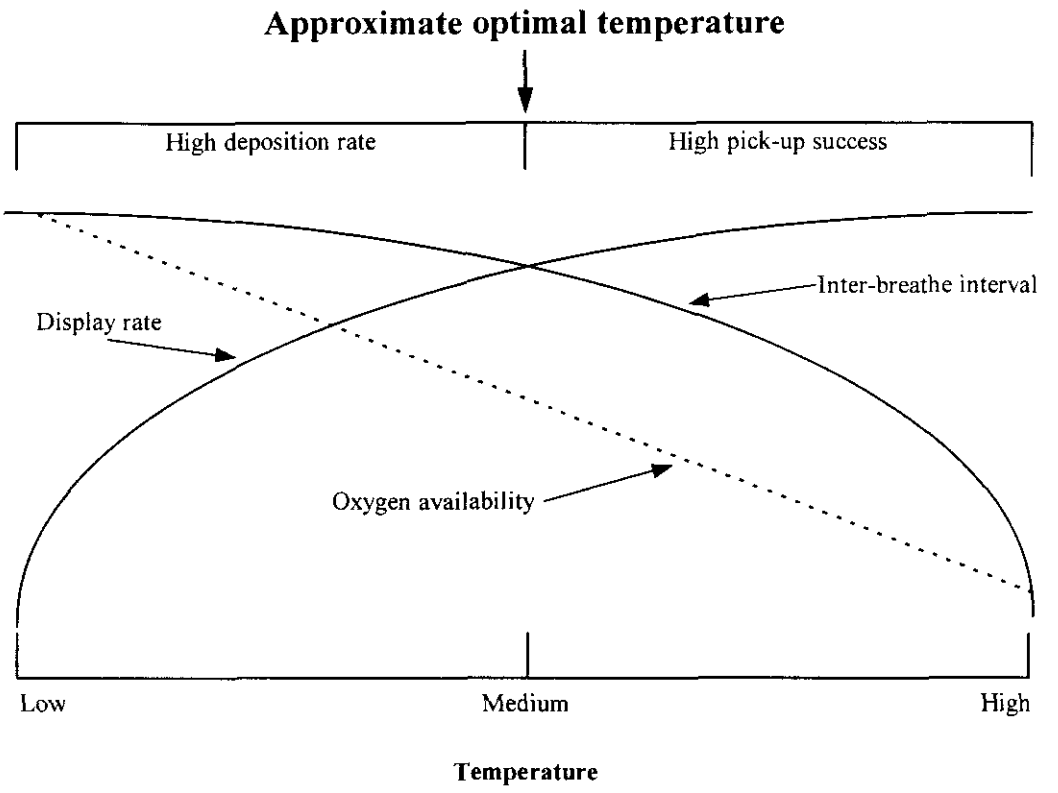


Figure 2.9. Summary of the effect of temperature on courtship: laboratory experiments.

The folly of extrapolating results obtained from specific systems in the laboratory to the functioning of the whole animal in its natural environment has been consistently stressed in the literature (Huey and Stevenson 1979; Huey 1982; Gatten et al. 1992; Hutchison and Dupré 1992; Rome et al. 1992). In the present study, for example, the excessively low display rate, long inter-breathe intervals and high deposition scores obtained at the low temperature (experiment two) were all unnatural consequences of the experimental design which required males to continue courting until they needed to breathe. In addition, encounters containing five or more sequences, frequently obtained at high temperatures in the laboratory (experiment one), are unlikely to be repeated in the wild (an important fact to bear in mind when considering the results from other experiments, carried out at room temperature). In the next chapter, I move on to a more natural environment and look at the effect of temperature in a semi-natural population of newts.

Chapter three

The effect of temperature: observations of a semi-natural population

3.1 Introduction

In Chapter two, the physiological constraints imposed by temperature on the courtship of smooth newts were considered, using data from laboratory experiments. In this chapter, I build on this physiological framework through a more ecological approach, using a longitudinal study of a semi-natural population of *Triturus v. vulgaris*.

Sexual activity has been investigated in relation to many factors including life-history constraints (Berven 1982; Partridge and Endler 1987), physiological constraints (Halliday 1987), and the conflict between predator avoidance and courtship behaviour (Ryan et al. 1982). The relationship between environmental factors and sexual activity has also been studied in terms of the timing of breeding, some species exhibiting considerable local adaptation in different parts of their geographical range. For example in Brittany, the common frog, *Rana temporaria,* spawns in January, in Finland at the end of May, and at an altitude of 2000m in the Alps in July (Beattie 1985). In the North of England, Beattie showed that spawning was delayed by 18 days for every 1°C drop in mean winter soil temperature.

The association between environmental factors and variation in sexual activity *within* the breeding season has been less well documented, an exception being the research into the effect of temperature on anuran calling behaviour (Pough et al. 1992) and on anuran chorus attendance (Henzi et al. 1995). Since newts are ectothermic and live in a variable environment, the short-term effect of such factors are potentially very significant.

Griffiths (1985) studied the daily activity pattern of smooth newts in relation to fluctuations of temperature and light (field experiments) and endogenous rhythms (laboratory experiments). The light-dark cycle was the primary controller of diel activity with sexual behaviour being limited to a bimodal (dawn and dusk) rhythm which coincided with a narrow band of illuminance. In the field, however, this pattern was modified by temperature, low

morning temperatures severely depressing the dawn peak and warm afternoon temperatures enhancing the dusk peak in sexual activity. Most sexual activity therefore took place at dusk when maximum temperatures coincided with optimal light conditions.

On a daily basis, therefore, newts appear to be sexually active when temperatures are most advantageous. Does this apply to the breeding period as a whole? Do individuals use all of the long season for breeding or do they maximise sexual activity when conditions are environmentally favourable? Conclusions from the experiments in Chapter two suggested that at low temperatures (early spring) courting pairs should experience low spermatophore deposition rates, while at high temperatures (early summer) there should be a reduction in pick-up success. I therefore set out to test the hypothesis that *maximum courtship activity in smooth newts takes place when periods of optimal temperatures (approximately 12 – 14°C) occur within the season.* If this is correct, then sexual activity and the opportunity for sexual selection. is concentrated into specific periods within the breeding season.

In Chapter one, however, a longitudinal laboratory experiment was described (Hosie 1992) in which half the inseminations took place within the first week of testing, the remainder being spread over the rest of the season. If this situation were mirrored in the wild, it would conflict with the use of optimal temperatures outlined above.

Although highly desirable, there are intrinsic problems associated with the study of aquatic urodeles in the wild, since most are active at dusk in murky, weed-filled ponds. A number of field studies of *Triturus* species have been carried out (for example, Harrison et al. 1983; Verrell and Halliday 1985a) but few have included observations of courtship behaviour. Notable exceptions are Griffiths (1985), Hedlund (1990) and Verrell and McCabe (1988). Verrell and McCabe clearly illustrate the difficulty with observing courtship behaviour in the wild: in two years, only 39 sexual encounters were seen of which just four included spermatophore deposition.

If data gathering is difficult in the wild, the opposite is true of laboratory experiments from which results are readily forthcoming (for example, Halliday 1977b; Verrell 1986b; Green 1991a; Waights 1996). However, conditions in the laboratory often bear little resemblance to those in the field and the lack of environmental complexity can make the interpretation of results difficult (Verrell 1989a; Sullivan et al. 1995) and even irrelevant. For example, Verrell (1982a) described 'hula', a type of courtship behaviour in *Notophthalmus viridescens* seen in the laboratory, but which was not observed in the field (Massey 1988). In a review of the sexual strategies of urodeles, Verrell (1989a) advocated a dual approach, using both laboratory and field data to achieve a better understanding of sexual behaviour.

I have added a third strategy which forms a useful compromise: a semi-natural environment, in which a population of newts is kept undisturbed and out of doors, whilst remaining clearly visible. Results from observation of the semi-natural population over two breeding seasons forms the basis of the next two chapters. This approach allowed me to analyse the relationship between specific activities and temperature (this chapter), social interactions between individuals and variation in mating success (Chapter four). The longitudinal approach enabled me to investigate the constraints imposed by the breeding season itself.

Aim: ***to investigate the effect of temperature on sexual and non-sexual activity over the breeding season.***

The purpose of this study was to observe free-living newts in a natural situation. Within the general hypothesis outlined above it fell under the category of exploratory rather than experimental research, and therefore did not involve the setting up of more specific hypotheses (Jaeger and Halliday in prep.). Nevertheless, the following questions clarify the aims I had in mind.

Specific questions:

1. *How does courtship behaviour in a semi-natural situation compare with findings from laboratory experiments? In particular, does more courtship take place under environmentally favourable conditions?*
2. *To what extent do various sexual and non-sexual activities depend on temperature?*
3. *What proportion of time is spent in sexual activity, compared with non-sexual activity?*

While newts were being collected from the pond at Braystone Farm, a number of temperature measurements were made at the Source pond and adjacent ponds in order to record the variation in water temperature over the season. Temperature measurements were also taken at the Open University (OU) field site pond. Since this is relevant to the subject of this chapter, these results will also be analysed and discussed here.

3.2 Methods

3.2.1 Apparatus

A 2 × 2m fish-stock tank was floored with gravel and filled with water to a depth of 60cm. Bricks and broken flower pots were arranged in the centre to provide both hiding places and terrestrial sites for newts emerging at the end of the season. Two rows of bricks radiated from the centre to the edge of the artificial pond to break up the open area into smaller sections (Figure 3.1). Artificial weed (made from green plastic bags cut into toothed strips) was inserted in abundance between the bricks to provide more cover and egg-laying sites, while avoiding the loss of visibility caused by live weed and algae. The water was stocked with *Daphnia* during the autumn of 1993 and maintained a healthy population throughout the 1994 reproductive season. In 1995, *Daphnia* was not added until late winter and bloomed less successfully during the cold spring.

The artificial pond was lit by a halogen lamp from dusk to dawn, allowing habituation to the light and preventing sudden changes in illumination. Buchanan (1993) showed that sudden

changes in the light intensity (commonly associated with the use of torchlight for nocturnal observation) significantly reduced the foraging activity of nocturnal frogs. Use of torchlight in the field has also been reported to startle newts, often causing them to flee (Verrell and McCabe 1988). Since only half the pond was clearly lit, a Petzl head-lamp was used as a supplement during the periods of observation. There was no evidence that the newts preferred either the well-lit or the poorly-lit side of the artificial pond. The pond was divided by a string grid into eight outer sections and one central section (Figure 3.1). Newts in the central section were assumed to be hiding (inactive) and behaviour was recorded only in the outer quadrats, 1 – 8.

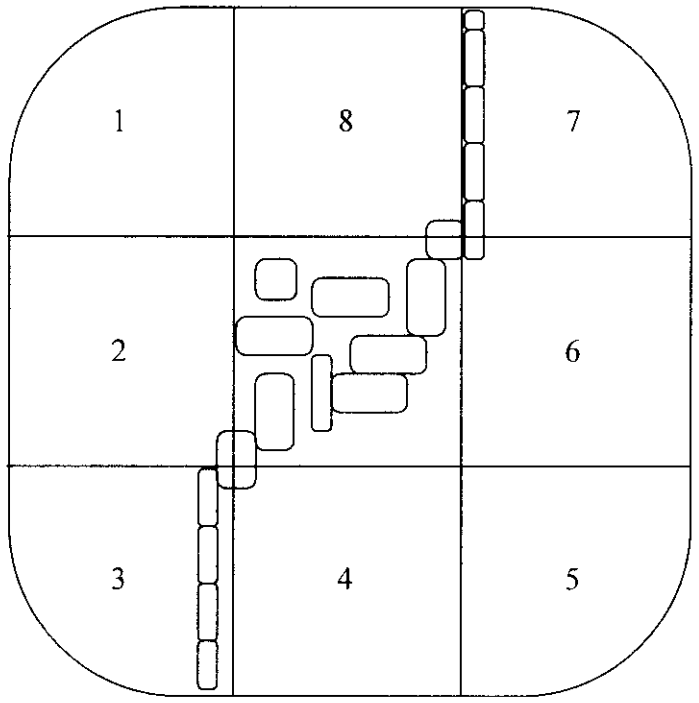


Figure 3.1. Diagram of the artificial pond showing the division into quadrats and the arrangement of bricks (rectangles).

The identification of newts was achieved by toe-clipping, although recognition of individual markings became possible with increasing familiarity. Sex ratios in natural newt populations exhibit considerable temporal and spatial variation (Harrison et al. 1983; Chapter six). In this experiment, sex ratios were maintained at 1:1.5 or 1:1.6 (m:f) as recorded in the wild by Verrell and Halliday (1985a) and the animals were introduced gradually to mimic the arrival of newts at a natural pond (Table 3.1).

1994			1995		
Date	**Number of males**	**Number of females**	**Date**	**Number of males**	**Number of females**
15 March	6	9	**2 February**	4	5
17 March	2	3	**3 February**	2	3
22 March	2	3	**10 February**	2	5
			13 February	2	3

Table 3.1. Dates on which newts were introduced into the artificial pond.

In 1994, it was necessary to replace an escaped female with a new unmated female and I was struck by the subsequent upsurge in male sexual activity. In order to find out if this was a characteristic reaction and to see if mated females had the same effect, I added three mated and four unmated females during the remainder of the season. Females from the established population were removed so that the density and sex ratio remained approximately the same. No new females were added in 1995.

Temperature and oxygen readings were taken regularly during the observation sessions using a dissolved-oxygen meter/ thermometer, while maximum-minimum temperatures were recorded daily. However, oxygen readings from the dissolved-oxygen meter were highly inconsistent and in 1995, the Winkler chemical test was used instead. Throughout, priority was given to creating as natural an environment as possible and animals were only disturbed two or three times during the season for weighing and measuring.

3.2.2 Observations

Observation took place between 15 March and 1 June, 1994 and between 2 February and 1 June, 1995, three to five times a week for approximately three hours at a time. Initially, each session began 1.25 hours after lighting-up time since, following preliminary observations, this appeared to be the newts' most active period. Later, as the days grew longer, the animals were more active earlier in the afternoon and observations began 1.5 hours before lighting-up time, corresponding to the peak of activity observed by Griffiths (1985).

Two types of sampling methods were employed:-

Scan sampling

The artificial pond was scanned every 15 minutes and the behaviour of each individual within the artificial pond and its position in relation to other newts was recorded. Scan sampling is particularly useful for assessing the proportion of individuals engaged in specific activities at any given moment (Martin and Bateson 1993).

Focal sampling

Between scans, observations focused specifically on sexual behaviour. If sexual activity occurred simultaneously in different groups or pairs, it was necessary to choose which individuals to follow. Courtship, likely to lead to spermatophore deposition, was given priority and described in detail, while a note was made of any other sexual behaviour. It was very rare for more than one sexual encounter to reach retreat display at the same time. Focal sampling also took precedence over scan sampling if there was a possibility of spermatophore transfer. Sexual behaviour was recorded using a voice-activated tape-recorder.

3.2.3 Additional temperature and oxygen measurements

In 1994, temperature readings were taken from the Source pond and two adjacent ponds at Braystone Farm, and a recently dug pond at the Open University, all of which differed in terms of size, vegetation and shading (Table 3.2).

Name	Area (m)	% Emergent vegetation	% Submergent vegetation	% Shading
Source	10.0 × 6.4	15	18	35
Orchard	6.4 × 5.5	2	95	50
Bat	4.0 × 3.8	5	75	2
OU	5.5 × 2.3	0	55	0

Table 3.2. Details of the three ponds at Braystone Farm and the OU pond. Area was measured accurately but the other parameters were estimated by eye.

Temperature was measured (using the dissolved-oxygen meter) at the surface edge, surface centre, shelf and at depth (Table 3.3). Shelf refers to the shallow, sloping area near to the edge of the pond where newts are thought to court most frequently (Halliday pers. comm.: pers. obs.). Maximum-minimum thermometers were also placed on the shelf in the Source and

Orchard ponds. Dissolved oxygen concentration was measured at the surface edge of all the ponds.

Pond	Measurement	Surface edge (cm)	Shelf (cm)	Surface centre (cm)	Depth (cm)
Source	Distance from edge	70	70	350	350
	Depth	0	20	0	75
Orchard	Distance from edge	65	65	220	220
	Depth	0	18	0	65
Bat	Distance from edge	35	35	100	100
	Depth	0	23	0	55
OU	Distance from edge	20	20	120	120
	Depth	0	20	0	60

Table 3.3. Location of temperature measurements at the three Braystone Farm ponds and the Open University pond.

3.3 Analysis and results

3.3.1 Non-sexual activity (scan sampling)

During scan sampling, the following non-sexual activities were recorded:-

1. Stationary — Individuals were motionless but within quadrats 1 – 8.
2. 'Foraging' — Newts were crawling along the substrate or floating mid-water while hunting for *Daphnia*.
3. Swimming — Individuals moved purposefully in one direction (including to the surface to breathe).
4. Egg-laying — Females were wrapping eggs in the artificial weed.

Mean scan time was one minute 39 seconds with an average of eight scans per night in 1994 and four in 1995. In order to compare the relationship between activity and temperature, the total number of individuals engaged in each activity per night was divided by the number of scans to give a mean number of individuals per scan. This number was expressed as a percentage of the total population.

The relationship between temperature and non-sexual activity is illustrated in Figure 3.2 and Figure 3.3. All the following graphs of temperature and activity (both non-sexual and sexual) represent only those days on which observation took place, but on no occasion was there a gap of more than two days.

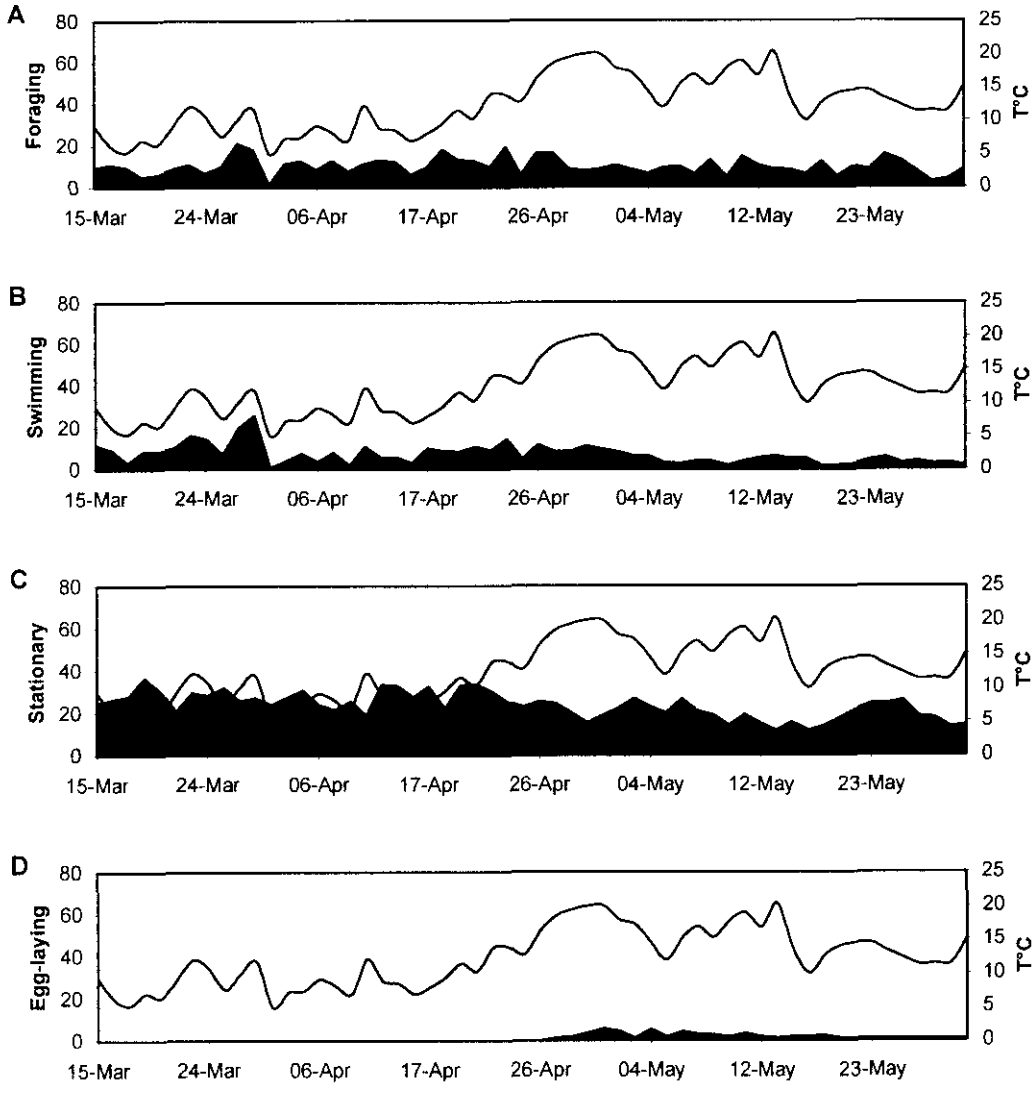


Figure 3.2. Temperature and components of non-sexual activity, 1994. Shaded area (left-hand y-axis): mean percentage of individuals engaged in each activity per night. Line (right-hand y-axis): temperature.

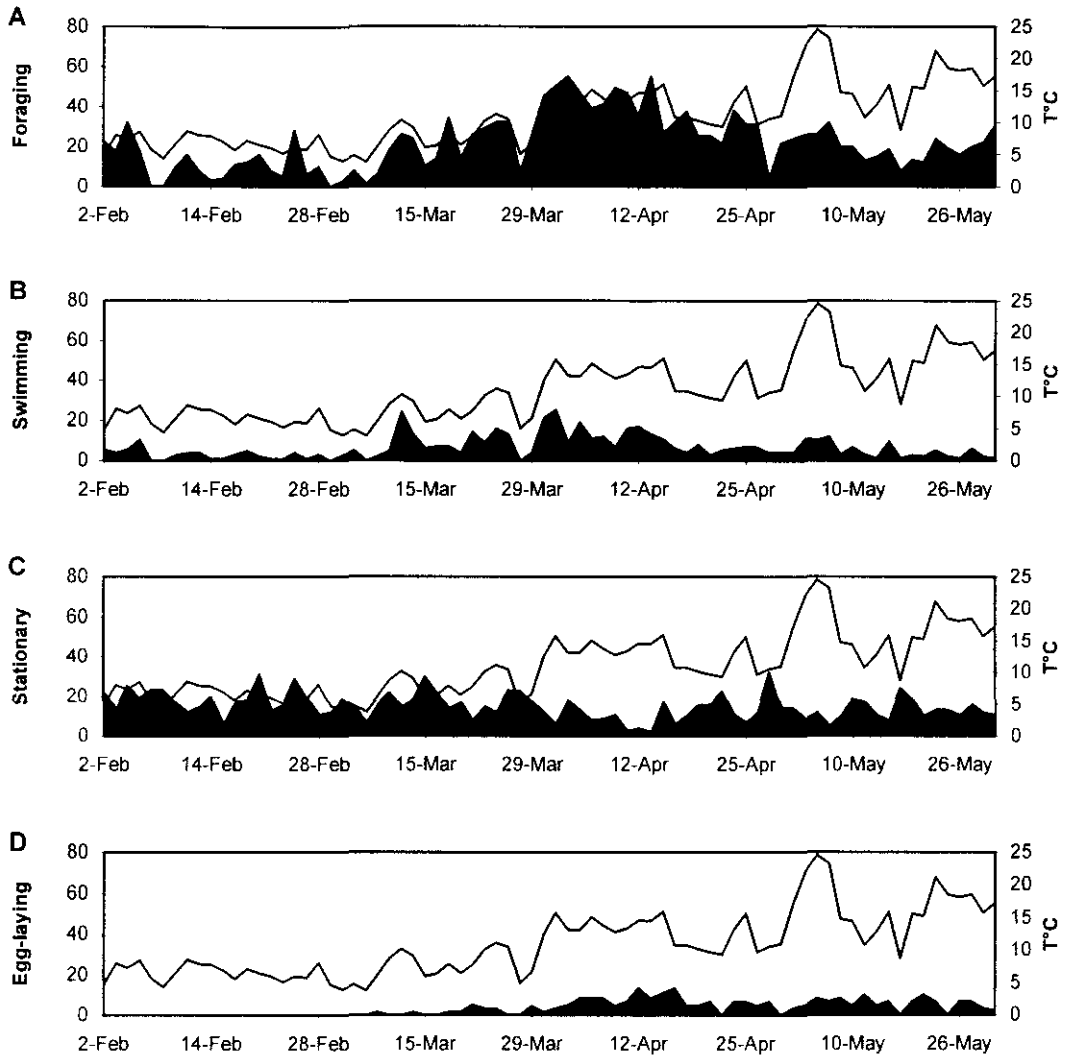


Figure 3.3. Temperature and components of non-sexual activity, 1995. Shaded area (left-hand y-axis): mean percentage of individuals engaged in each activity per night. Line (right-hand y-axis): temperature.

After examining the data by eye, it seemed appropriate to divide the season into two halves for the purpose of correlation. Following the approximate midpoint (26 April, 1994 and 9 March, 1995) temperature remained consistently above about 10°C and (with the exception of egg-laying) there was no longer any association between temperature and activity.

Spearman rank-order correlations were used to examine the statistical relationship between

foraging, swimming or stationary individuals and temperature during the first half of the season, and between egg-laying and temperature during the second.

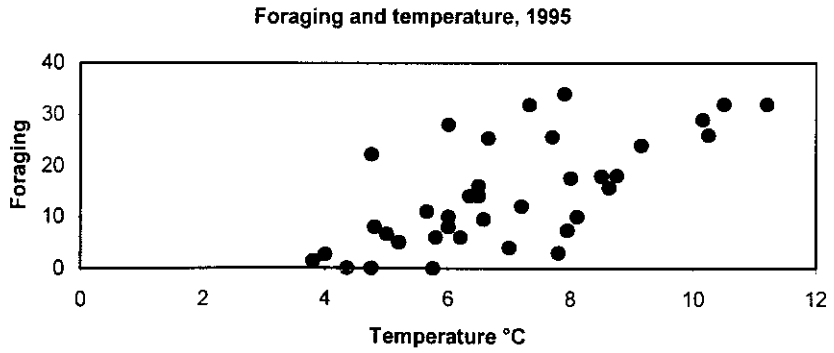
In both years, foraging was significantly correlated with temperature during the first part of the season (Figure 3.2A, Figure 3.3A and Statistics box 3.1) but whereas the number of foraging individuals remained fairly constant in 1994, there was a marked increase during the warm period 29 March – 25 April in 1995. It is possible that the poor bloom of *Daphnia* in 1995 forced the newts to spend more time in search of food.

Statistics box 3.1

Spearman rank-order correlations between temperature and non-sexual activity (scan sampling). Tests are two-tailed.

1994	**Date**	**n**	r_s	**p**
Foraging	15 March – 26 April	56	+0.39	**< 0.05**
Swimming	15 March – 26 April	56	+0.67	**< 0.001**
Stationary	15 March – 26 April	56	-0.05	> 0.05
Egg-laying	27 April – 1 June	27	+0.38	**< 0.05**
1995	**Date**	**n**	r_s	**p**
Foraging	2 February – 29 March	37	+0.68	**< 0.001**
Swimming	2 February – 29 March	37	+0.66	**< 0.001**
Swimming	28 February – 1 April	21	+0.84	**< 0.001**
Stationary	2 February – 29 March	37	-0.19	> 0.2
Egg-laying	18 March – 1 June	47	+0.38	**< 0.01**

The following graph of foraging and temperature (2 February – 29 March, 1995) provides a visual example of a typical correlation.



At the beginning of the 1994 season, the proportion of swimming individuals was also closely correlated with temperature. In 1995, however, swimming activity was at a minimum at the start of the season and only began to fluctuate with temperature after about 28 February (Figure 3.2B, Figure 3.3B and Statistics box 3.1). Observation began six weeks earlier in 1995, immediately after the first individuals had arrived at the drift fence when mean evening temperature was only 6.6°C (2 February to 15 March). Presumably, during the prolonged cold period, newts were reducing energetic costs to a minimum. In both years there was a decrease in swimming activity towards the end of the season which coincided with the peak of egg-laying, suggesting that some swimming activity had been directed towards the pursuit of females (although some of the decrease may be accounted for by the egg-laying females themselves).

The proportion of stationary individuals decreased as temperature rose consistently above about 10°C (Figure 3.2C and Figure 3.3C) but there was no relationship between small scale fluctuations in temperature and the number of stationary individuals (Statistics box 3.1). A weak correlation was found between temperature and the number of females laying eggs (Figure 3.2D, Figure 3.3D and Statistics box 3.1).

In summary, there was a close correlation between temperature, swimming and foraging at the beginning of the season, but this relationship weakened as temperatures rose above about 10°C. The number of stationary individuals did not fluctuate with temperature, but an overall decline coincided with the warmer part of the season. Temperature was weakly correlated with egg-laying during the second half of the season.

3.3.2 Sexual activity (focal sampling)

Courtship display was divided into categories, each of which referred to a single encounter of any duration, but which was terminated when all individuals moved apart and remained separated for a minimum of one minute.

1. OA — Orientation without display. Newts approached to within one body length of each other. This category included sniffing and chasing.
2. OD — Orientation with display. Any number of 'move-to-the-front', 'whips' or 'fans' were performed by the male but with no positive response by the female.
3. RD — The female responded positively to male display (by remaining still or approaching) but no spermatophore deposition occurred.
4. SP — Courtship reached the spermatophore transfer phase, resulting in deposition with or without pick-up.

Table 3.4 shows the total number of each type of encounter. Only 3% of all courtships between males and females reached the spermatophore transfer phase (SP). Courtships reaching retreat display but not continuing to the spermatophore phase (RD) were uncommon, suggesting that once a female started to approach, she was likely to continue to respond positively. In the following analyses RD was combined with OA + OD to represent all encounters without deposition.

	OA	OD	RD	SP
1994	625	537	12	39
1995	369	579	11	30

Table 3.4. Total numbers of each type of encounter.

For the purpose of relating sexual activity to temperature, the total number of each type of encounter (OA, OD, RD and SP) was calculated for every night of observation. This was divided by the observation time to give the number of encounters per hour. Changes in sexual activity over the season and the relationship between these changes and temperature are illustrated in Figure 3.4 and Figure 3.5. As already mentioned, the addition of new females to the artificial pond in 1994 had a significant effect on sexual behaviour which should be distinguished from that of temperature. The black dots mark the days on which a new female was introduced.

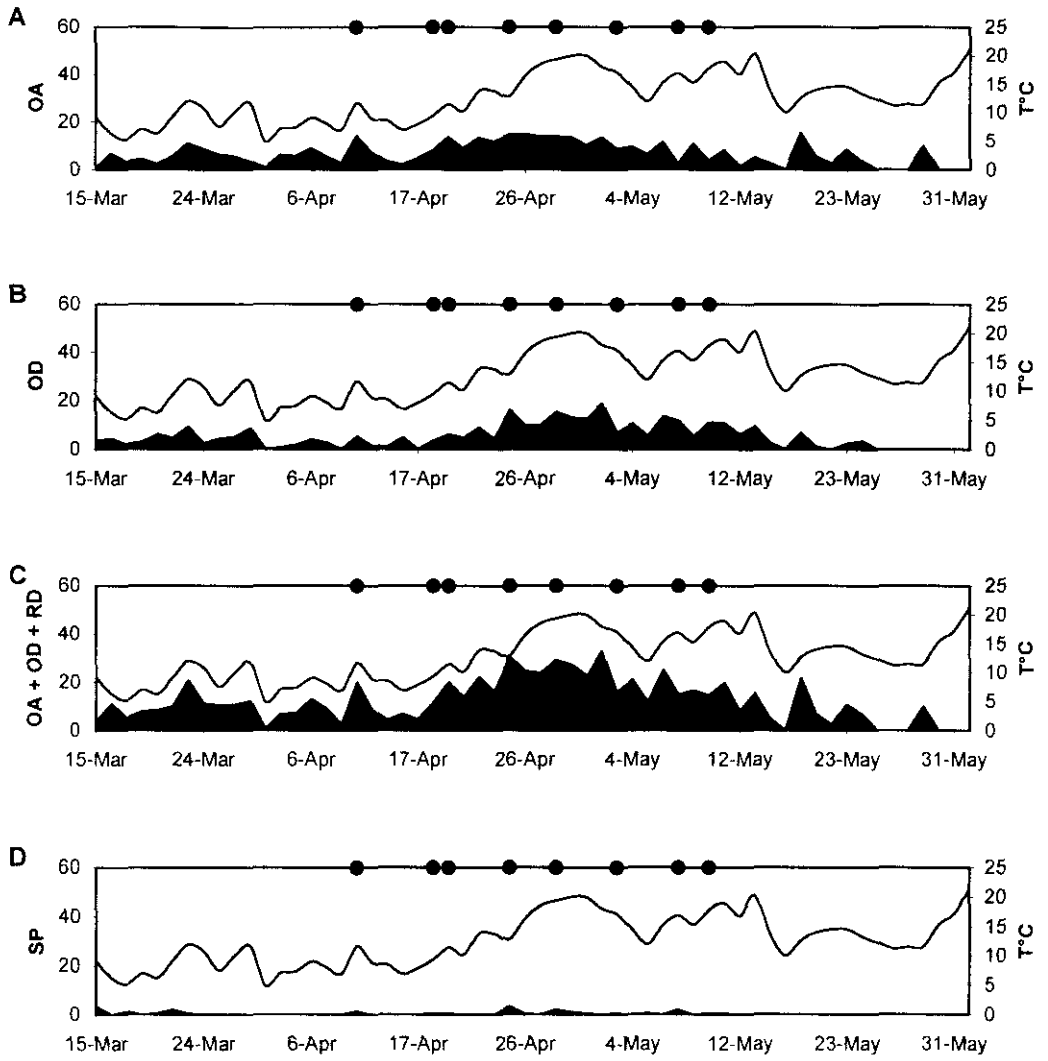


Figure 3.4. Temperature and components of sexual activity (focal sampling) 1994. Shaded area (left-hand y-axis): number of encounters per hour. Line (right-hand y-axis): temperature. Black dots mark the days on which a new female was introduced.

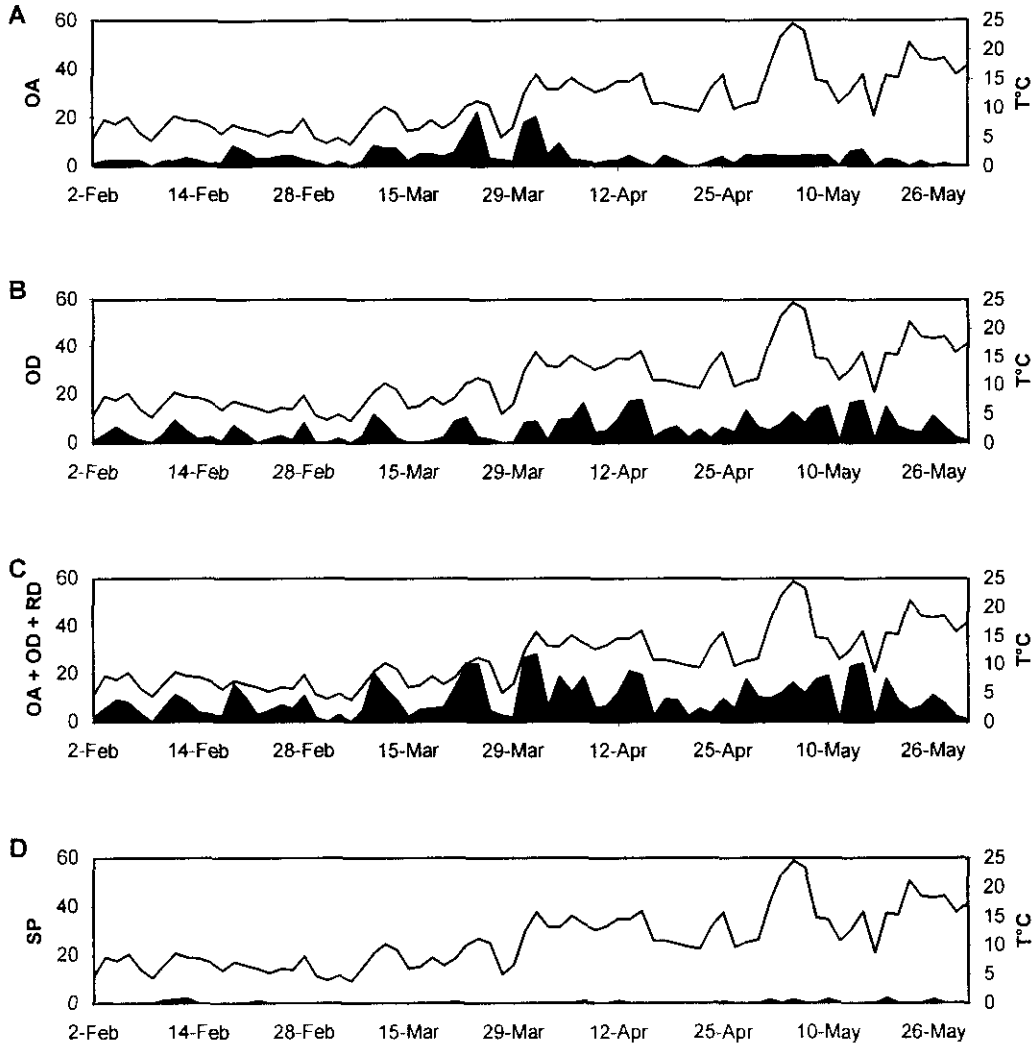


Figure 3.5. Temperature and components of sexual activity (focal sampling) 1995. Shaded area (left-hand y-axis): number of encounters per hour. Line (right-hand y-axis): temperature.

Results from 1994 and 1995 were broadly similar, despite different weather conditions, but there was some interesting variation between the two years. A brief examination of the data indicated that it was appropriate to use the same division of the season into two (26 April, 1994; 29 March, 1995) for the analysis of sexual behaviour. After these dates, temperatures remained above about 10°C and egg-laying began.

During the first part of the season, encounters without deposition (OA + OD + RD) were closely correlated with temperature (Statistics box 3.2) although relative proportions of orientation with and without display (OD and OA) varied between nights. The number of encounters without display was reduced during the initial cold period of 1995 when temperatures averaged only 6.6°C. This corresponded to the reduction in swimming activity recorded from scans during the same period. Courtship without display typically included long chases of unreceptive females. It appears that, at low temperatures, males tended to concentrate their efforts on females willing to allow display rather than waste energy in potentially fruitless pursuit.

During the second part of the season, the number of encounters without display increased but no longer followed fluctuations in temperature. Only sudden drops in temperature (for example 14 May, 1994) caused a concurrent drop in activity. The rise in sexual activity,

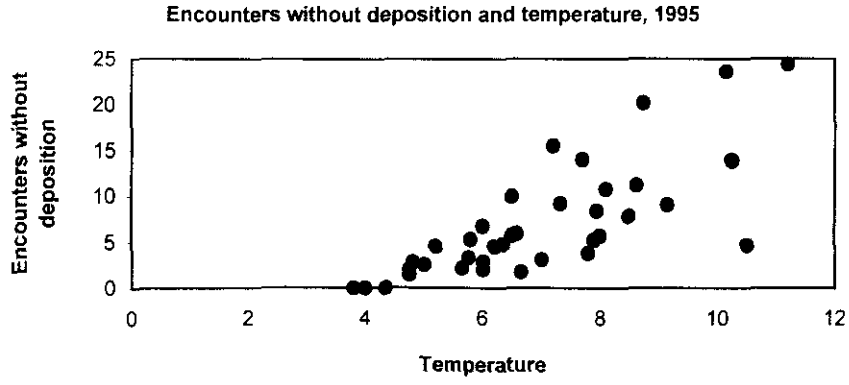
Statistics box 3.2.

Spearman rank-order correlations between temperature and encounters without deposition (focal sampling). Tests are two-tailed.

1994. 15 March – 26 April (n = 29): $r_s = +0.82$, $\mathbf{p < 0.001}$.

1995. 2 February – 29 March (n = 37): $r_s = +0.81$, $\mathbf{p < 0.001}$.

The following graph of encounters without deposition (2 February – 29 March, 1995) and temperature provides a visual example of the correlations.



however, was greater in 1994 than 1995, possibly due to the prolonged nature of the 1995 season.

The most striking result from the focal study was the scarcity of encounters reaching the spermatophore transfer phase. After a few days at the beginning of the season in which high levels of deposition coincided with the arrival of the population, there was a considerable time lapse before courtship with deposition was observed again. Most remaining spermatophore transfer occurred after the onset of egg-laying.

It was not possible to carry out a correlation analysis between encounters with deposition (SP) and temperature because of the predominance of days with no deposition (zero scores). In order to overcome this, temperature was divided into low (≤10°C), medium (10.1 – 16°C) and high (>16°C). The number of days with and without encounters reaching the spermatophore transfer phase were then calculated within each of the three temperature ranges and the G-test was performed (Table 3.5).

Year	Temperature	Days without deposition	Days with deposition
1994	**Low**	15	5
	Medium	19	5
	High	6	8
1995	**Low**	31	6
	Medium	25	5
	High	6	3

Table 3.5. Spermatophore deposition and temperature. Number of days with and without encounters reaching spermatophore transfer within three temperature ranges.

There was no significant difference in the proportion of days with and without encounters reaching spermatophore transfer within the three temperature ranges (Statistics box 3.3). However, during the first few days of the 1995 season (when mean evening temperatures at the artificial pond were often as low as 4 – 5°C), no spermatophore transfer occurred at temperatures below 6°C. In 1994, observations began later and temperatures averaged 8.3°C over the first five days on which deposition took place (although one of these was as low as 5.2°C). It seems reasonable to propose 6°C as a threshold below which spermatophore transfer does not usually occur.

In summary, the correlation between encounters without deposition and temperature indicates that (below about 10°C) the intensity with which males attempted to court females varied with temperature. On the other hand, the willingness of females to mate (encounters with deposition) appeared not to be governed by temperature above a threshold of approxmately 6°C.

3.3.3 Sexual activity (scan sampling)

Orientation with and without display (OD and OA) was also recorded during scan sampling. The mean number of individuals per scan involved in sexual activity was calculated in the same way as non-sexual activity (section 3.3.1) and the recipients of display (whether responsive or not) were included in the count since they were prevented from performing other activities (Figure 3.6).

Statistics box 3.3

G test to compare the number of days with and without deposition within three temperature ranges (focal sampling).

1994. $G = 5.66$, $df = 2$, $p > 0.05$.

1995. $G = 1.38$, $df = 2$, $p > 0.3$.

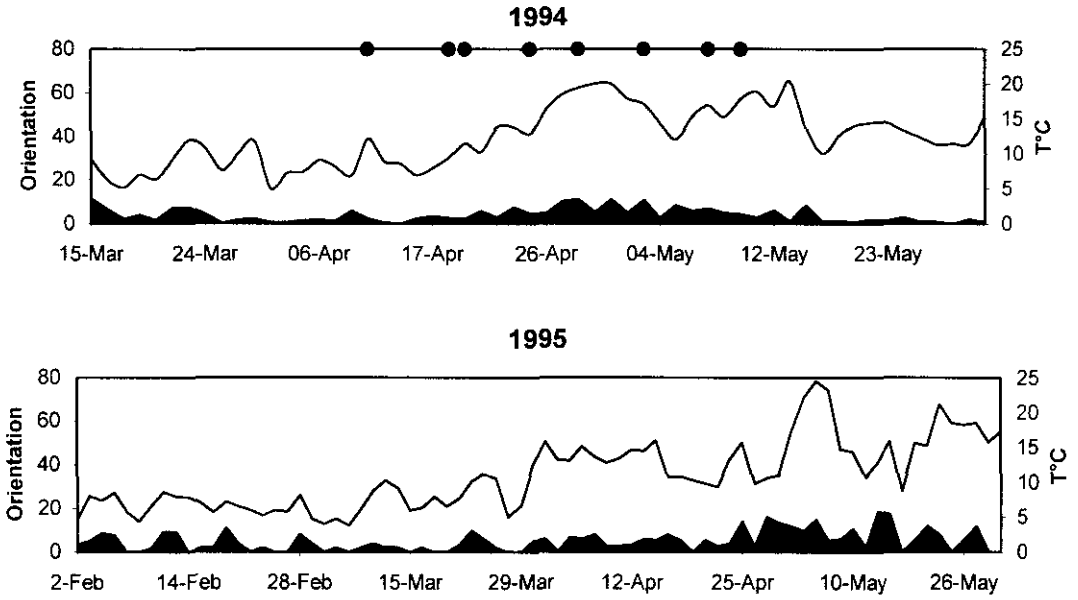


Figure 3.6. Temperature and components of sexual activity (scan sampling). Shaded area (left-hand y-axis): mean percentage of individuals engaged in sexual activity per night. Line (right-hand y-axis): temperature. Black dots mark the days on which a new female was introduced.

Overall, the relationship between orientation and temperature corresponded closely to that obtained from the focal study and once again, there was a significant correlation during the first half of the season (Statistics box 3.4).

Since both sexual and non-sexual activity were recorded during scan sampling, the proportion of time occupied by each could be compared. The percentage of individuals engaged in sexual and non-sexual activity each night was calculated within the three temperature bands (low ≤ 10°C; medium 10.1 – 16°C; high > 16°C) and since the results were not normally distributed, median values were used to summarise the data (Table 3.6).

Statistics box 3.4

Spearman rank-order correlations between temperature and orientation (scan sampling). Tests are two-tailed.

1994. 15 March – 26 April (n = 56): $r_s = +0.37$, $\mathbf{p < 0.05}$.

1995. 2 February – 29 March (n = 37): $r_s = +0.54$, $\mathbf{p < 0.001}$.

Year	Temperature	n	Non-sexual activity (median %)	Sexual activity (median %)	Total
1994	**Low**	20	44	3	47
	Medium	24	38	3	41
	High	14	36	7	43
1995	**Low**	37	32	2	34
	Medium	30	52	8	60
	High	9	42	7	49

Table 3.6. Median percentage of individuals engaged in non-sexual and sexual activities each night within three temperature ranges. n: number of days.

Between 30 and 60% of the population were visible on the substrate at any one time and the majority were engaged in non-sexual activity. Only 2 – 8% exhibited sexual behaviour at any one time, the smallest percentage occurring lowest at low temperatures. If only 3% of sexual encounters result in deposition (Table 3.4) and approximately less than 10% of the population are involved in courtship at any one time, the incidence of spermatophore transfer in the wild is clearly very low.

3.3.4 Multiple regression

In 1994, a series of multiple regression analyses were carried out to investigate the influence of other climatic variables on sexual activity. The following variables were obtained from Cranfield Airport and Bracknell Meteorological Office, Woburn Station, both of which are within a few miles of the study site:-

Cranfield: evening and mean daily dry bulb temperature, wind speed (knots), cloud cover and atmospheric pressure.

Woburn: maximum daily temperature, minimum daily temperature, mean wind speed, rainfall and sunshine hours.

In addition, the differences in temperature and pressure from the previous day were calculated.

Since it is not appropriate to use independent variables which are highly correlated in multiple regression (Zar 1984), it was necessary to choose from amongst the alternative data sets for each type of climatic measurement. In order to do this, evening and mean variables from Cranfield and all variables from Woburn were analysed separately with the number of encounters without deposition (OA + OD + RD) and those producing the most significant

partial regression coefficients were selected for use in further analysis. These were:- Cranfield: mean cloud cover (CrM Cl); difference in evening temperature from previous day (CrE T diff); evening wind speed (CrE wind); evening pressure (CrE P); Woburn: rainfall (W rain); sunshine hours (W sun). Water temperature from the artificial pond (Wt) was also included in the analysis. Stepwise multiple regression was carried out using the Linest function in Microsoft Excel (Table 3.7). All variables were included in the first analysis. The t-statistic was then determined for individual variables by dividing the partial regression coefficient by the standard error. Variables with a significant t-statistic were used in the second regression.

	r^2	F	df	p	Wt	CrM Cl	CrE T diff	CrE wind	CrE P	W rain	W sun
All days	0.34	3.51	47	< 0.01	**1.01** **0.28**	-1.39 0.85	-0.10 0.37	**0.42** **0.19**	0.07 0.14	-0.42 0.54	-0.28 0.34
All days (2 vars.)	0.29	10.4	52	< 0.001	**1.10** **0.36**			0.36 0.18			
to 26 April	0.74	8.34	21	< 0.001	**2.07** **0.32**	-1.30 0.78	0.06 0.29	0.14 0.15	0.05 0.10	-0.23 0.36	-0.12 0.28
to 26 April (1 var.)	0.72	60	27	< 0.001	**2.13** **0.27**						

Table 3.7. Multiple regression of encounters without deposition (OA + OD + RD) and seven selected climatic variables. R^2: coefficient of determination, F: the F statistic, df: degrees of freedom. The coefficient and standard errors are given for each independent variable. Variables in bold had a significant t-statistic (see text).

The F-statistic, which determines whether the apparent relationship between the independent and dependent variables occurred by chance, was significant in all four regressions (Table 3.7) but the relationship between sexual activity and climatic variables improved when the second part of the season was omitted. Water temperature accounted for 72% of the variation in the number of encounters without deposition until 26 April. No other variable influenced sexual activity in the artificial pond. This analysis emphasised the importance of water temperature, adding weight to the laboratory experiments described in Chapter two.

3.3.5 Deposition and pick-up

Figure 3.7 shows cumulative plots of spermatophore deposition and pick-up during 1994 and 1995.

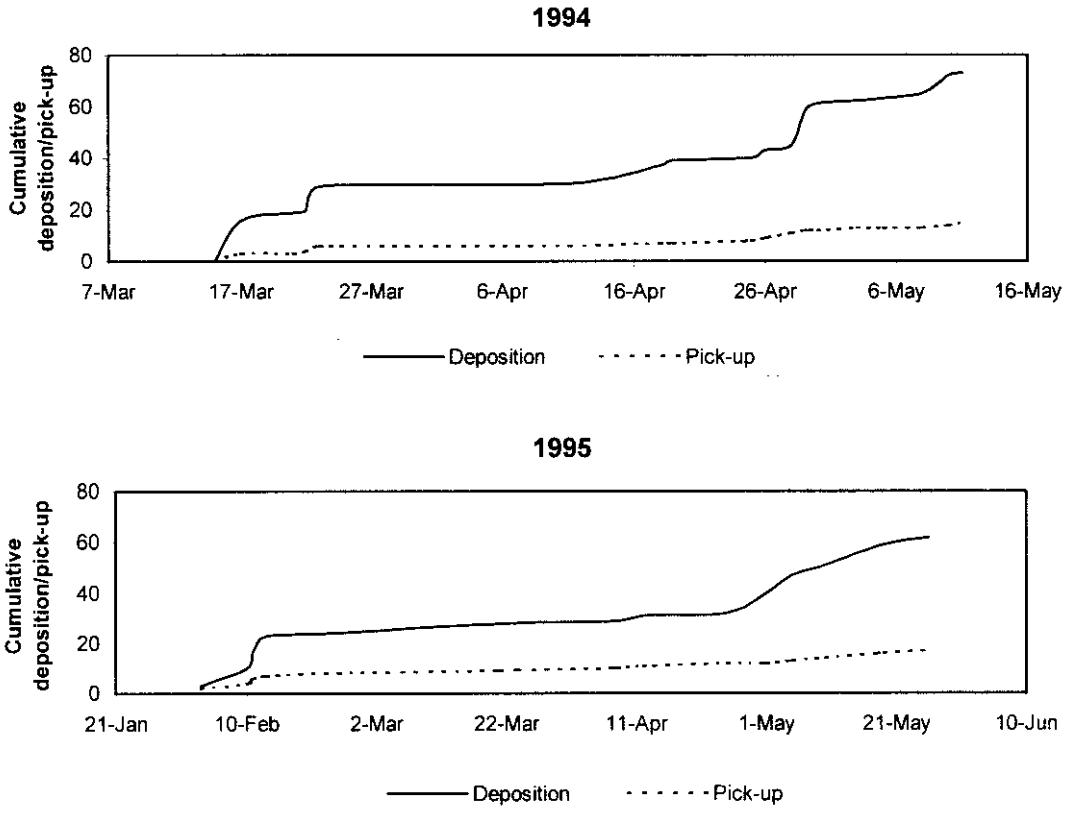


Figure 3.7. Cumulative deposition and pick-up over the season.

In both years, about 40% of spermatophore depositions and pick-ups occurred during the introduction of the original population (approximately one week). This was followed by occasional depositions until late April in 1994 and the beginning of May in 1995 when deposition rate increased again. In 1994 this coincided with the beginning of egg-laying and in 1995 with the middle of the egg-laying period (refer back to Figure 3.2D and Figure 3.3D). It is important to stress the imprecise nature of these figures. The number of pick-ups was a conservative estimate since on occasions when the spermatophore was not visible, it was recorded as a miss. Moreover, additional depositions and pick-ups undoubtedly took place while the population was not being observed.

In 1994, new females were occasionally added to the population. 32% of spermatophores were deposited for these (both mated and unmated) (Table 3.8).

	1994 including new females	1994 excluding new females	1995
Total number of depositions	75	51	62
Total number of pick-ups	16	12	17
% pick-up	21	23.5	26.2

Table 3.8. Spermatophore transfer in the artificial pond.

In 1994, overall pick-up success was 21% (16/75) but only four (16%) of the 24 spermatophores deposited for new females were picked up, suggesting that males were highly stimulated by these females and tended to deposit very readily. The effect of unfamiliar females on males was studied in detail in a laboratory experiment (Chapter five).

Temperature was again divided into three categories (low ≤ 10°C; medium 10.1 – 16°C; high > 16°C) in order to compare results from the artificial pond with the laboratory data, described in Chapter two (Table 3.9 and Figure 3.8).

		Courtships	Depositions	Median depositions per encounter	Pick -up	% Pick-up
1994 with new female	**Low**	17	32	1	6	20.7
	Med	7	14	1	3	21.4
	High	15	29	1	7	21.8
1994 without new female	**Low**	16	30	1	6	21.4
	Med	5	8	1	2	28.6
	High	9	13	1	4	28.6
1995	**Low**	15	26	1	9	32.1
	Med	9	16	1	6	37.5
	High	6	17	1	2	11.1
Laboratory	**Low**	16	47	3	32	68
	Med	18	64	3.5	43	67
	High	18	63	3.5	29	46

Table 3.9. A comparison of spermatophore transfer between the artificial pond and the laboratory (Chapter two).

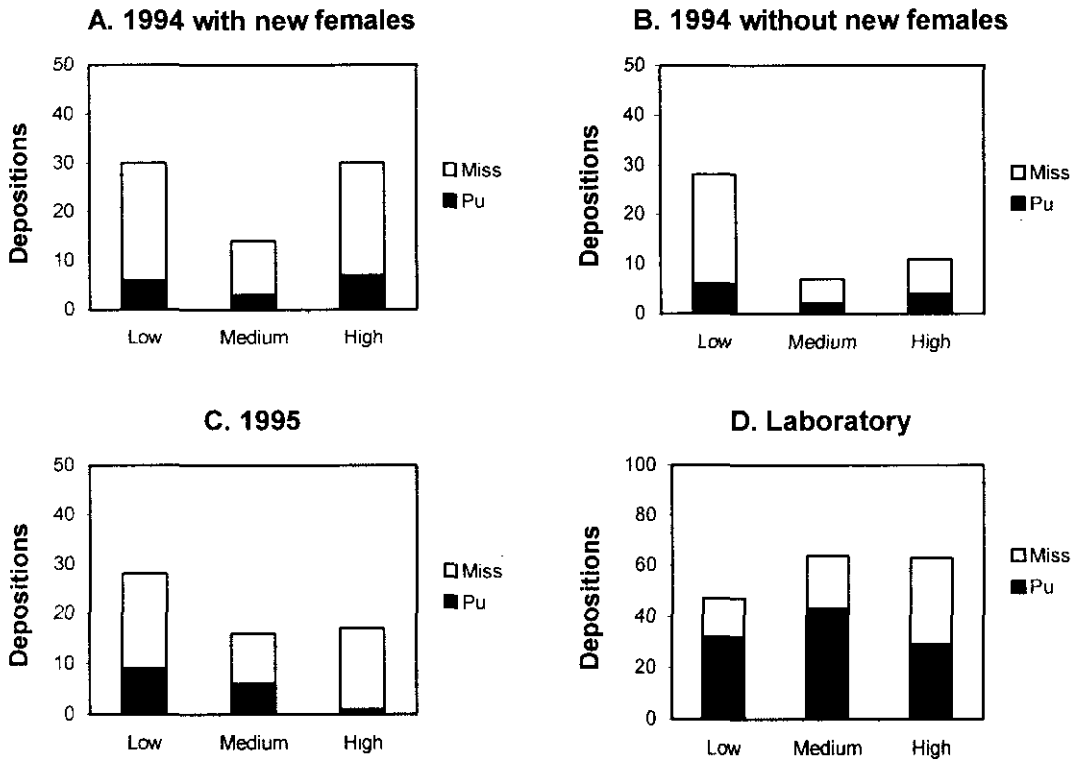


Figure 3.8. A comparison of spermatophore transfer between the artificial pond and the laboratory (Chapter two). Note the different scale for the y-axis in the laboratory. Pu: pick-up.

In the laboratory experiment, spermatophore transfer was shown to be most successful at the medium temperature, the result of both high deposition and pick-up rates. This was contradicted by results from the artificial pond in two respects. First, there were many depositions at low temperature, reflecting the period of arrival of the initial population, whereas at the medium and high temperatures deposition was less frequent, unless new females were introduced. Secondly, the median number of depositions per encounter was considerably reduced in the artificial pond. There was also a marked difference in pick-up success between the artificial pond and the laboratory, an average of only 25% compared with 60% respectively. In the artificial pond, pick-up success varied little between temperatures, with the exception of the high temperature in 1995, when the low percentage pick-up did correspond to results obtained from the laboratory.

3.3.6 Additional temperature and oxygen measurements

Natural variation in spring temperature and oxygen readings within and between ponds was obtained by comparing measurements from Braystone Farm, the OU field site and the artificial pond.

Figure 3.9 illustrates temperature readings, taken from different areas in the pond, using the Source pond as an example. To eliminate the effect of time of day, I have only included days on which temperature was measured between 7:00 and 8:00am (n = 42). 'Surface centre' readings were remarkably similar to those from the shelf and have been omitted for clarity.

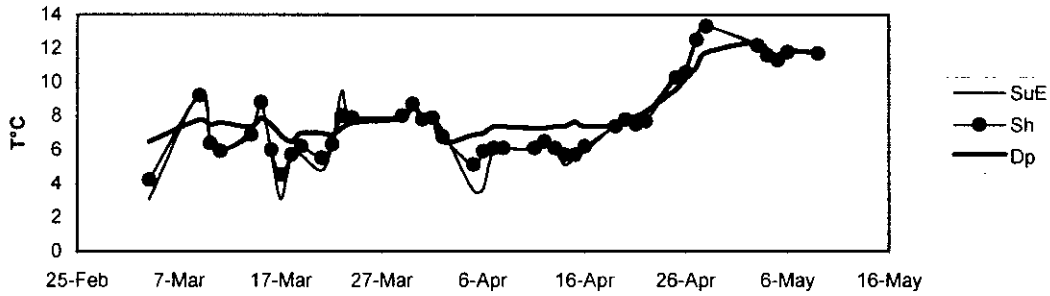


Figure 3.9. Temperatures at different parts of the Source pond over the season, 1994. SuE: surface edge. Sh: shelf (depth 18 – 22cm). Dp: depth 55 – 75cm.

Surface edge readings, where water is in contact with air, were the most variable and fluctuated above and below the depth measurements, depending on the amount of morning sunshine. Depth readings were the most constant, averaging about 7.5°C during the first part of the season and rising to 10°C during April. The shelf (and surface centre) readings were intermediate. fluctuating less than those at the surface edge. In comparison with the Source pond, temperature differences were smaller in the more sheltered Orchard pond and larger in the small, deep and exposed Bat pond (Table 3.10).

	Difference between surface edge and depth		Difference between surface edge and shelf	
	Maximum °C	**Mean °C**	**Maximum °C**	**Mean °C**
Source	3.3	1.1	2.2	0.3
Orchard	2.2	0.4	1.5	0.2
Bat	3.4	1.7	2.6	0.8

Table 3.10. Maximum and mean differences in temperature between different parts of three ponds.

Newts, however, court most frequently at dusk and the temperature profile of a pond is often reversed at this time of day. Dusk temperatures could not be obtained from the ponds at Braystone Farm but were measured at the Open University pond (Figure 3.10).

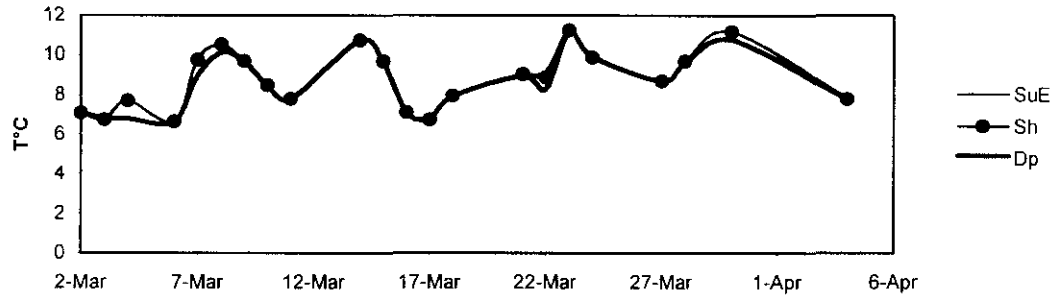


Figure 3.10. Mean evening temperatures at different parts of the OU pond during the early season, 1994. SuE: surface edge. Sh: shelf. Dp: depth. (Surface edge and shelf measurements were too similar to be distinguished on the graph).

In this small, shallow pond, pond depth and surface temperatures were almost identical in the evening. However, the difference between surface and depth readings will increase with the size and depth of the pond. John Tyler kindly allowed me to examine temperature readings from a larger pond at the Sevenoaks Wildfowl Reserve which contains a population of smooth newts (Figure 3.11). The pond measures 7 × 4m + 7 × 3m with 75% submergent vegetation, 10% emergent vegetation and 5% shading. Depth measurements were 60cm below the surface. Surface temperatures clearly exceeded those at depth during the afternoon and early evening.

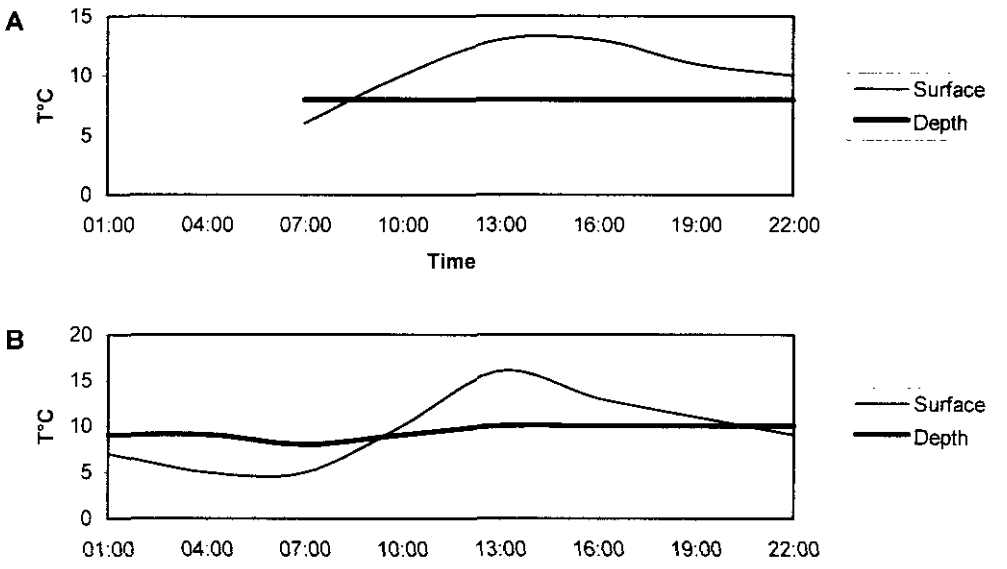


Figure 3.11. Surface and depth temperatures as a function of time of day. A: 14 March, 1994. B: 26 March, 1994. Data with kind permission of John Tyler, Sevenoaks Wildfowl Reserve.

Oxygen levels at Braystone Farm, the Open University pond and the artificial pond are shown in Figure 3.12. The Source and Orchard pond displayed similar levels of oxygen, fluctuating between about 4mg/l and 8mg/l throughout the season. These levels are considerably lower than those under which the breathing experiment (Chapter two) was conducted (8mg/l – 11mg/l).

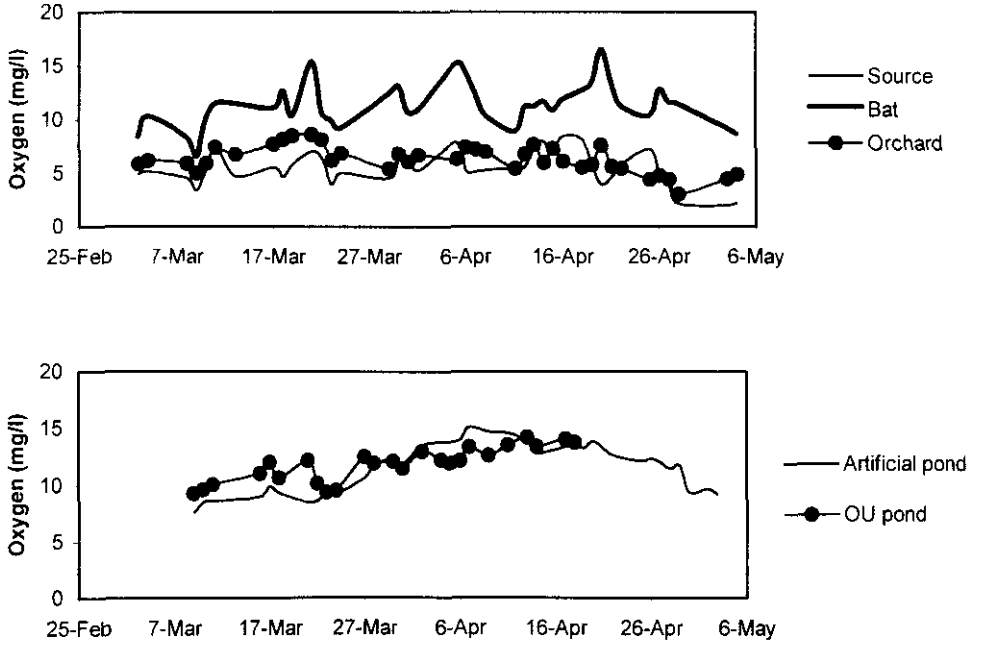


Figure 3.12. Oxygen measurements, 1994.

The Bat pond exhibited higher but widely fluctuating amounts of dissolved oxygen (7mg/l – 14mg/l), closer to the levels seen in the Open University pond and artificial pond which were low in weed as well as invertebrate and vertebrate life. Oxygen levels may have been high in the Bat pond because it is deep relative to the small surface area. These oxygen readings encompass those from the experiment in Chapter two. There was no obvious decline in oxygen levels as temperatures rose during the season. Clearly the relationship between temperature and oxygen in a natural environment will be affected by the complex balance between photosynthesising and respiring organisms. The Winkler tests for dissolved oxygen carried out in 1995 confirmed the earlier readings, using the dissolved-oxygen meter.

3.3.7 Summary of results from Chapter three

Non-sexual activity

1. The amount of swimming and foraging was correlated with temperature during the first part of the season when temperatures were usually below about 10°C.

2. As temperatures rose, the number of individuals engaged in each activity no longer reflected each fluctuation. Foraging remained constant under conditions of saturated food supply but increased during the warmer months when food availability was limited.

3. The number of stationary individuals was not related to the short term fluctuations in temperature but decreased overall with rising temperatures.

4. Egg-laying was weakly but significantly correlated with temperature.

Sexual activity

1. Only 3% of encounters reached the spermatophore transfer phase.

2. During the first half of the season, there was a significant correlation between temperature and the number of encounters without deposition (OA + OD + RD).

3. As temperatures rose during the second part of the season, the number of encounters without deposition increased considerably in 1994 and slightly in 1995. This period coincided with the peak of egg-laying.

4. In contrast, the number of courtships reaching spermatophore transfer did not appear to be governed by temperature, although deposition rarely took place below 6°C.

5. Approximately half the population was visible during each scan and only 2 – 8% of individuals exhibited sexual behaviour.

6. Water temperature was the only climatic variable to influence sexual activity.

Deposition and pick-up

1. At least 40% of all depositions and pick-ups occurred within the first week.

2. Patterns of spermatophore transfer did not corroborate results obtained in the laboratory. An initial spate of depositions coincided with the arrival of the population when temperatures were low. During the remainder of the season (at medium and high temperatures) courtship with deposition was sporadic, occurring mostly during the egg-laying period or following the introduction of a new female. Pick-up success was markedly lower in the artificial pond than the laboratory and, with the exception of the high temperature in 1995, was fairly constant across all temperature ranges.

Temperature and oxygen

1. Water temperature varied between different parts of the ponds and with the time of day.

2. At the shallow edge of the pond (the site of most courtship) morning temperatures were variable while evening temperatures tended to equal or exceed those at depth.

3. In general, oxygen levels were lower in the well-established ponds, containing abundant plant and animal life, than in the newly-dug or artificial ponds.

3.4 Discussion

Although smooth newts spend several months in the water during spring and early summer, it is clear that a relatively small portion of their aquatic phase involves sexual activity. Results from the scan sampling showed that, during the evening, less than 10% of individuals were sexually active at any one time, and the majority of these did not complete courtship. Only 3% of encounters reached the deposition stage. A comparison with field studies of *Triturus* and other genera from the family Salamandridae (Table 3.11) indicates that a similar scarcity of successful encounters has been widely reported and suggests that this is not an artefact resulting from problems of observation.

Species	Study period	Total encounters	Spa	% Spa	Authors
T. v. vulgaris	134 days over 2 seasons	2202 (1208 with display)	69	3 (6)	Kauffmann (this thesis)
T. v. vulgaris	65 days over 2 seasons	63	4	6.3	(Verrell and McCabe 1988)
T. v. meridionalis	2 seasons (days not specified)	57	3	6	(Pavignano et al. 1993)
T. cristatus	50 days over 4 seasons	200 (54 > 30s)	4	2 (7)	(Hedlund 1990)
T. cristatus & *T. marmoratus*	68 days over 1 season	185	20*	?	(Zuiderwijk and Sparreboom 1986)
T. italicus	Not specified	74	21*	?	(Giacoma and Crusco 1987)
T. boscai	34 days over 2 seasons	315	21	7	(Faria 1995)
Notophthalmus viridescens	2 seasons (days not specified)	131	11	8.3	(Massey 1988)
Cynops ensicauda	11 days over 1 season	100	4	4	(Sparreboom and Ota 1995)

Table 3.11. Field data showing the proportion of sexual encounters leading to spermatophore deposition. Spa: encounters with deposition. *: figure represents the number of depositions rather than the number of encounters. *Cynops ensicauda*: subspecies *popei*.

The number of courtships reaching deposition was extremely low in all the above studies, ranging from 2 – 8% (mean 5.1%) if all encounters are included and 4 – 8% (mean 6.4%) if courtships without display and courtships less than 30 seconds in length are omitted (studies by Kauffmann and Hedlund). The percentage of courtships containing pick-up would be even lower. Pick-up success was estimated by Faria (1995) as between 16% and 36% of depositions

(similar figures to 22% from the semi-natural population) while Sparreboom and Ota (1995) and Giacoma and Crusco (1987) recorded figures of 50% (2/4 depositions) and 9.5% (2/21 depositions) respectively. All authors agree that the low proportion of courtships with deposition is the result both of females being unreceptive during many courtships and of interference by other males (Chapter four).

3.4.1 A longitudinal profile of the breeding season

The longitudinal nature of this study has been invaluable in providing information about the response of smooth newts to changing conditions during the season. Figure 3.13 gives an overview of the relationship between sexual behaviour, non-sexual activity and temperature during the breeding season.

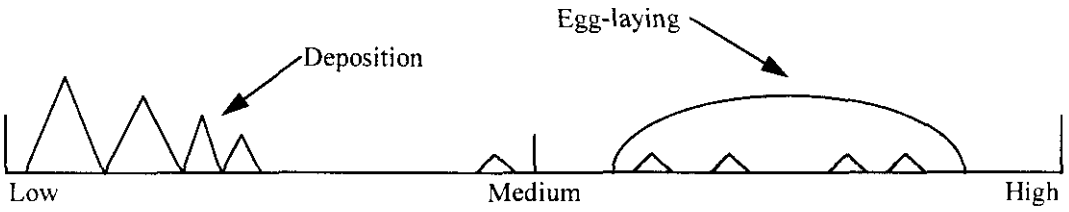


Figure 3.13. Longitudinal profile of events within the breeding season and their relationship with temperature.

During the early part of the season, cold temperatures were associated with a reduction in the number of individuals engaged in swimming, foraging, and sexual activity (scan sampling) and the amount of courtship without deposition (focal sampling). These activities closely mirrored fluctuations in temperatures, correlations being particularly high in 1995 (which had lower than average temperatures over this part of the season). At low temperatures, males also

abandoned the pursuit of unreceptive females and concentrated on those willing to allow display.

Thus activities which are dependent on effective locomotion (non-sexual and male sexual behaviour) were affected by temperatures below about 10°C. In the laboratory experiment (Chapter two) there was a marked increase in the effect of temperature on display rate between the medium and low temperatures (12 – 14°C and 6 – 8°C) and 10°C may therefore represent the point below which locomotion begins to be inhibited by temperature.

In the second half of the season, the response to temperature was less sensitive and more variable. Some activities increased overall with the rising temperatures (male sexual activity) while others decreased (swimming, stationary) and yet others remained constant (foraging). Two characteristics distinguished this part of the season: temperature remained consistently above about 10°C and females began to lay eggs.

Water temperature appears to be the only climatic variable affecting courtship in the smooth newt. This is perhaps not surprising, considering that courtship is wholly aquatic in this species. A range of climatic variables have been shown to affect other species of amphibian in which breeding activity occurs at terrestrial or semi-terrestrial sites. For example, approximately 77% of the variation in chorus attendance by male *Hyperolius marmoratus* could be explained by a mixture of climatic variables, in particular those associated with preventing water loss by evaporation (Henzi et al. 1995). The natterjack toad, *Bufo calamita*, has a prolonged breeding season during which periods of calling and spawning can be accurately predicted by a combination of climatic variables, including minimum temperature above 5°C for the preceding three or four nights (Banks and Beebee 1986).

In contrast to male sexual behaviour, female receptivity to courtship (manifested by encounters reaching deposition) did not respond to changes in temperature, although

deposition rarely took place below 6°C. What other factors could influence the timing of femle receptivity?

The pattern of deposition recorded during both years of observation corresponded closely to data from Hosie (1992) with at least 40% of inseminations occurring during the first few days. The mean oviposition period per female is 37 days (Baker 1992a) although a large female may need as much as 90 days to complete egg-laying (Halliday pers. comm.). It is therefore likely to be adaptive for females to start egg-laying as early as possible in the season (Hosie 1992).

Recent research has shown that the smooth newt is polyspermic, with an average of four (range 1 – 20) sperm entering each egg (Waights 1998). Although one spermatophore, containing 38,000 – 148,000 sperm (Waights 1998) is still theoretically sufficient to fertilise a complete clutch of eggs, sperm may not remain viable for the three month egg-laying period (Baker 1992a; Pecio 1992). Pecio (1992), for example, demonstrated that some females begin to lay unfertilised eggs between five and 39 days after initial insemination, but lay fertilised eggs again following re-insemination. In addition, spermiophagy has been demonstrated in female *Triturus italicus*, late in the season although this has yet to be confirmed in *T. vulgaris* which was investigated at the start of the season only (Sever et al. in press). In this study (after the intial peak of deposition) most successful courtships occurred between mid-April and mid-May during the peak of egg-laying. The need for additional or fresher sperm may therefore influence the timing of female receptivity, later in the season. Temperature will have an indirect affect since it is positively correlated with the amount of egg-laying activity (Hosie 1992, this study).

In the introduction to this chapter, I put forward the hypothesis that *maximum courtship activity in smooth newts takes place when periods of optimal temperatures (approximately 12 – 14°C) occur within the season.* This is clearly incompatible with the pattern of female

receptivity outlined above and the hypothesis can be rejected. Since females were highly receptive at the beginning of the season, a large proportion of inseminations, of necessity, took place at low temperatures (between 6°C and 10°C). Deposition at the 'optimal' medium temperatures was uncommon because females tended to be unreceptive during periods when such temperatures occurred.

In two of the rare field studies of newt reproductive behaviour, Verrell and McCabe (1988, *Triturus vulgaris*) and Hedlund (1990, *T. cristatus*) also found that most mating activity occurred at the beginning of the season, following which females re-mated occasionally during the egg-laying period. These changes in female sexual behaviour throughout the season have a profound affect on the operational sex ratio and competition for mates (Verrell and McCabe 1988, Chapters four, five and seven). In this study, it was also shown that the introduction of new females (both mated and unmated) affected the amount of male sexual activity. The opportunities for males to court successfully will therefore depend not only on changes in female receptivity but also on the frequency with which novel females are encountered and the timing of female arrival (Chapters five and six).

3.4.2 Thermoregulation and acclimation

The temperature measurements described in this chapter indicate the extent to which smooth newts are able to cope with a variable thermal environment. Temperatures ranged from freezing to 30°C over a season and by as much as 20°C over the course of a day. Surface temperatures tended to be lower than deep water temperatures in the morning and higher in the evening, although the thermal profile depended to a large extent on the morphology and aspect of the pond.

Smooth newts mostly court at the shallow edges of ponds where early evening temperatures tend to be most favourable (although the newts may also be responding to appropriate light levels in the shallows, Griffiths 1985). This can therefore be interpreted as

thermoregulatory behaviour. In comparison with the sophisticated themoregulation of fish and reptiles, amphibians are generally considered poor thermoregulators. Recent research, however, has revealed both behavioural and physiological mechanisms for controlling temperature in amphibians (Hutchison and Dupré 1992). In the aquatic environment, the high heat capacity and conductivity of water means that body temperature is rarely more than 2°C above or below the surrounding water (Schmidt-Nielson 1990; Spotila et al. 1992) and behavioural thermoregulation consists of movement to and from warmer or cooler areas (Brattstrom 1979). In a field study of the American salamander, *Ambystoma tigrinum*, Whitford and Massey (1970) observed thermoregulatory behaviour at temperatures below 15°C, the animals moving to warmer shallow waters during the day while movement became random at temperatures above 15°C.

Acclimation (usually applied to experimental manipulation) and acclimatisation (occurring in the field) have identical meanings based on the premise that the past thermal experience of the individual will improve its current performance. For an amphibian in cold conditions this would mean that, after a period of exposure to the cold, physiological capabilities would return to the levels associated with warmer temperatures. In the laboratory experiments (Chapter two), the newts were not given sufficient time to acclimate to their test temperatures and it is possible that performance in the artificial pond was improved following acclimatisation.

I calculated display rate in the same way as described in Chapter two, (measuring the interval between whip – whip, whip – fan and fan – whip) for all courtships without sexual interference which took place below 9.5°C. Results were pooled from a number of different pairs over the two years because there were insufficient data to obtain median scores for individuals (Figure 3.14). This allowed an approximate comparison with Figure 2.2 (Chapter two), reproduced here for convenience.

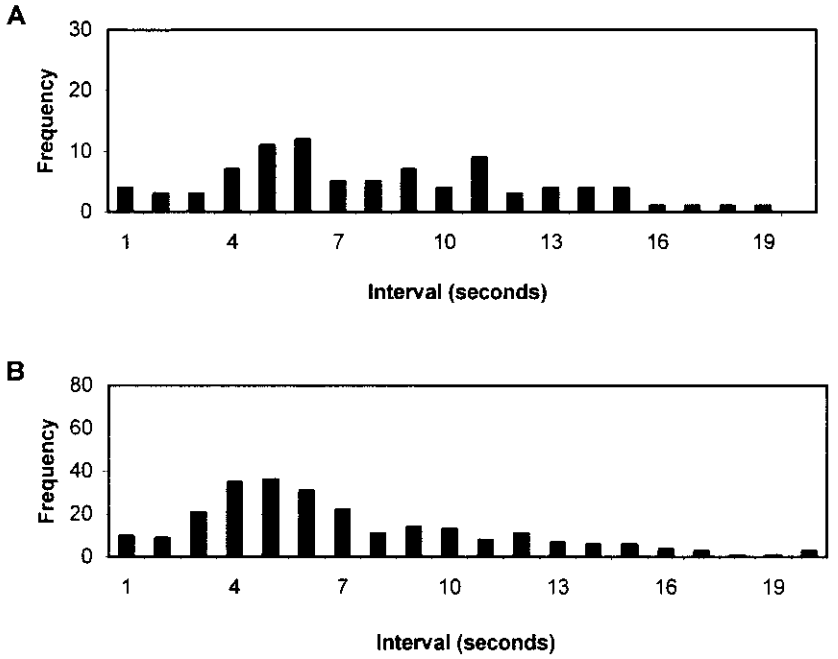


Figure 3.14. Frequency distribution of the intervals between display events at low temperatures. A: data from the artificial pond, 1994 and 1995. B: data from the laboratory experiment, 1994 (Chapter two).

The distributions were remarkably similar indicating that males did indeed display at a slow rate at low temperatures in the wild and there was no evidence that locomotion was improved through acclimatisation.

For this comparison to be valid, it was important to see if display rates also increased at high temperatures in the wild. Using the same procedure, I combined data from courtships at medium and high temperatures during the 1994 and 1995 observations and compared this with the laboratory data from the high temperature condition (Figure 3.15). Once again the distributions match very closely. The differences in display rate obtained during laboratory experiments appear to be a real effect which also occurred under more natural conditions.

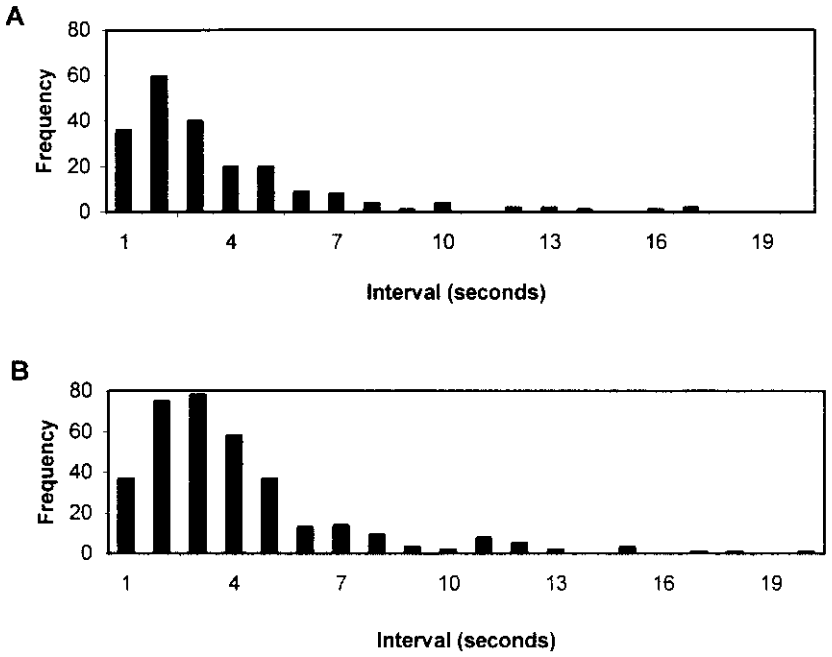


Figure 3.15. Frequency distribution of the intervals between display events at medium and high temperatures. A: data from 1994 and 1995 observations of the artificial pond. B: data from 1994 laboratory experiment (Chapter two).

The literature on acclimatisation is full of contradictions and is difficult to interpret since the results come from different species, temperatures and performance measurements (Gatten et al. 1992) but in general, amphibians appear to be limited in their ability to acclimate (Rome et al. 1992). Else and Bennett (1987) found no evidence for acclimation in *Ambystoma tigrinum nebulosum*, locomotor performance and muscle contractile function remaining temperature sensitive. These authors concluded: 'Thus behavioural performance is significantly impaired by low body temperatures, even those at which many biologically important activities, such as reproduction occur. From an adaptive perspective, these results are puzzling, considering the importance of these locomotor activities to survival and reproduction.' Pocrnjic (1965) on the other hand, found evidence for inverse compensation in *Triturus vulgaris*. In this type of acclimation, metabolic rate is further reduced at low temperatures in order to conserve energy over a period in which, for example, food availability may be scarce (Rome et al. 1992). Clearly, although this is important for long term survival, it does not facilitate vigorous activity such as courtship display at low temperatures.

It is widely accepted that low temperatures can severely limit behavioural capacities of amphibians (Bennett 1990). Returning to the quotation from Else and Bennett (1987) above, it is interesting to differentiate between the effect of temperature on activities needed for survival and those essential to reproduction. Whereas survival involves the acquisition of a minimum amount of food (an absolute measure), reproductive success depends to a large extent on *relative* competitive ability. In the case of smooth newts, males compete directly for females and all competitors therefore display at the same temperature. Differences in individual success will arise from variation in display ability within, rather than between, temperatures (Chapter four). In this study, spermatophore transfer was less sensitive to temperature than other activities, occurring above a minimum threshold of 6°C. Courtship may be have been successful at low temperatures because, at the beginning of the season, highly receptive females require minimal stimulation to reach the spermatophore transfer phase (Hosie 1992).

Temperature may have a greater impact on the timing of migration to the breeding pond (Chapter six) than on the level of sexual activity within the breeding season. As mentioned in the introduction, a number of species exhibit local adaptation to climatic conditions and *Triturus* species belong in this category. In Central Norway (the most Northern part of its range) the period of egg-laying in *Triturus vulgaris* begins later than in Britain (between May and June) and larval growth lasts well into September (Dolmen 1983). Dolmen does not report the timing of courtship but it must have occurred during the month of April when temperatures still only reached about 5°C. In Britain, *Triturus cristatus* enters the water shortly before *T. vulgaris*, usually early to mid February (pers. obs.) whereas in Sweden, Hedlund (1990) observed *T. cristatus* in late March, migrating across the snow to ponds which were still sometimes covered in ice. Clearly there are circumstances under which amphibians can be active in very cold conditions!

3.4.3. A comparison with laboratory data

How do the results from this chapter compare with those from the experiments from Chapter two? The differences in display rate between temperatures recorded in the laboratory, were mirrored in the semi-natural situation (Figure 3.14 and Figure 3.15, section 3.4.2 above). However, the optimal temperature for spermatophore transfer (12 – 14°C) obtained in the laboratory was not reflected in results from the artificial pond for two main reasons. Firstly, successful courtship appeared to be influenced by the pattern of female receptivity rather than by periods of favourable temperature. As a result, a large proportion of inseminations took place shortly after the arrival of the population even though temperatures were low. Secondly, the number of depositions per encounter and percentage pick-up were considerably reduced at all temperatures. Competition between males (absent from the laboratory environment) may be an important factor in this respect (see Chapters four and five).

The second laboratory experiment investigated the potential conflict between courtship and the reduction in dissolved oxygen at high temperatures. It was emphasised that such conflict existed only if spermatophore transfer was reduced by the need for increased pulmonary respiration. There was no evidence for this in the artificial pond since spermatophore deposition was equally rare at all temperatures. However, the dissolved oxygen levels at the Braystone Farm ponds were considerably lower than those seen in the laboratory and artificial pond and more work is needed to see if these levels, combined with high display rate, affect courtship at high temperatures in the wild.

These results have vindicated the plea by a number of authors (for example, Huey 1982; Rome et al. 1992) to put results from the laboratory into an ecological context. Although laboratory experiments are useful for determining physiological capabilities or optima, field studies are essential for understanding the constraints under which the whole animal operates. Hutchison and Dupré (1992) observed that amphibians are frequently active in their natural environment under conditions in which it is impossible to attain the preferred temperature. In

the case of the smooth newt it appears that there is little opportunity for courtship to take place at optimal temperatures.

3.4.4 Conclusions

As suggested by Verrell and McCabe (1988), the semi-natural population of smooth newts did not use the whole of their prolonged breeding season for sexual activity and only a small proportion of the population were involved in courtship at any one time. Although sexual activity and therefore the opportunity for sexual selection is thus concentrated into specific periods within the breeding season, these periods appear to be determined by the pattern of female receptivity rather than by favourable environmental conditions. As a result, courtship with deposition occurred most frequently at the beginning of the season when temperatures were less than optimal. In the short term, social factors, such as the arrival of novel females and competition between males may have greater influence on the success of courtship than environmental factors (Figure 3.16).

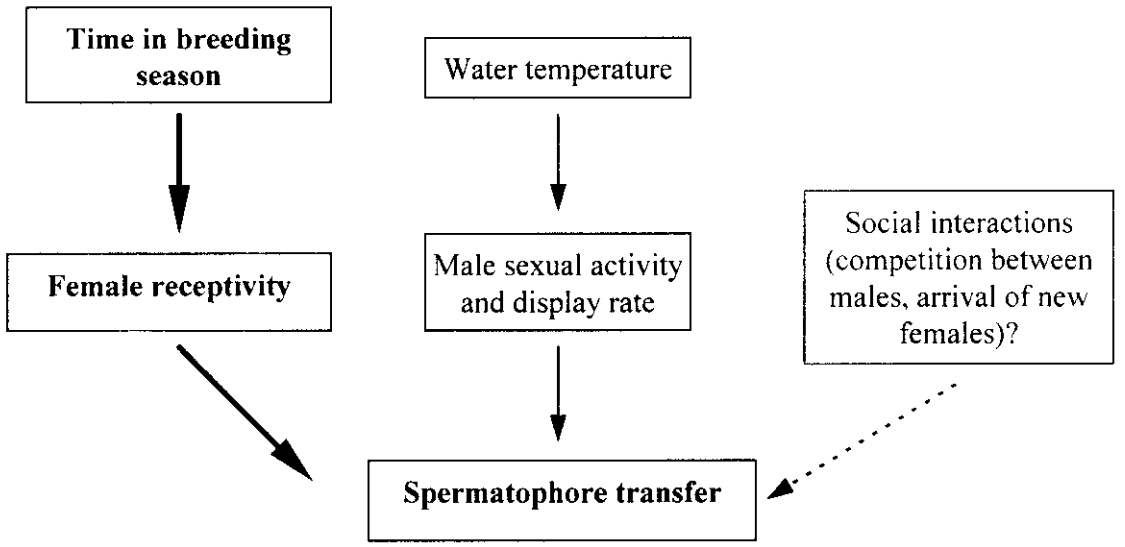


Figure 3.16. A summary of Chapters two and three: factors affecting spermatophore transfer in the smooth newt. Bold indicates a stronger link. Dotted arrow indicates that more information is needed.

The present study made two particular contributions to research on smooth newt courtship behaviour. The longitudinal approach illustrates the importance of constraints imposed by the breeding season itself and emphasises the range of conditions experienced by temperate species with prolonged breeding seasons. A combination of 'quick and easy' scan sampling and the more intensive focal sampling was used to monitor behaviour, and a remarkably good match between data on male sexual activity was obtained from both methods. Scan sampling would not normally be considered suitable for relatively infrequent events of this type, since the chance of scoring the event within any given moment in time is remote (Martin and Bateson 1993). Nevertheless, despite the fact that sexual activity only occurred in 2 – 8% of the population during a scan, this was frequent enough to be used successfully and scan sampling is therefore a useful tool for gauging the amount of male activity over an evening. Spermatophore transfer, on the other hand, would not generally be detected during scans.

The study of a semi-natural population provided information not available to either field or laboratory studies and is a useful tool for bridging the gap between these opposite approaches. The next chapter continues with the analysis of results from the semi-natural population, concentrating on social interactions between individuals and on individual variation in mating success.

Chapter four

Social factors: further analysis of data from the semi-natural population

4.1 Introduction

The first two data chapters dealt exclusively with the effect of environmental factors, in particular temperature, on smooth newt courtship and until now, I have ignored the nature of competition between individuals and the extent of individual variation in mating success. Using data obtained during observation of the semi-natural population, I investigate these aspects of sexual activity.

4.1.1 Reproductive rate, the operational sex ratio and sexual interference

Bateman (1948) first put forward the idea that the amount of investment in gamete production (large, nutrient-rich eggs versus small, nutrient-poor sperm) was important in explaining the tendency for females to be discriminating in their choice of mate and for males to compete for access to mates. Whereas female reproductive success is limited by the number of gametes she can produce, male reproductive success is limited by the number of inseminations he can obtain. In a landmark paper, Trivers (1972) extended Bateman's ideas to include all aspects of parental investment, such as nurturing and defending offspring as well as gamete production. He defined parental investment as 'any investment by the parent in an individual offspring that increases the offspring's chance of surviving (and hence reproductive success) at the cost of the parent's ability to invest in other offspring'. The sex which invests more is the limiting sex.

Emlen and Oring (1977) subsequently introduced the key concept of the *operational sex ratio* (OSR) defined as 'the average ratio of fertilizable females to sexually active males at any given time.' This provided the first quantitative method of estimating the potential for intra-specific competition for mates with the more numerous sex competing for the limiting sex. The OSR is not only influenced by the amount of parental care invested by each sex, but also

by the adult sex ratio and the spatial and temporal distribution of the sexes (Clutton-Brock and Vincent 1991; Clutton-Brock and Parker 1992). For instance, there will be limited opportunity for competition between males in populations in which all or most females become receptive simultaneously (Emlen and Oring 1977).

More recently, Clutton-Brock and Vincent (1991) defined the *potential rate of reproduction* as 'the maximum number of independent offspring that parents can produce per unit time'. They suggested that this is more easily measured than parental investment and gives a more accurate estimation of the OSR. In particular, there are species in which males are solely responsible for parental care and yet still compete for females, a situation not compatible with the parental investment theory. These males (usually ectotherms) achieve faster reproductive rates than females because, for example, they care simultaneously for multiple broods or for a short period of time per brood (Clutton-Brock and Vincent 1991).

At first glance, smooth newts fall into the classic scenario in which males contribute nothing but sperm to the reproductive process, while females invest not only in egg production but also in the time taken to wrap individual eggs in weed. The predicted outcome is a male-biased OSR with competition between males and discriminating females. However, there are additional factors which increase the complexity of this simple scenario.

First, arrival at the breeding site is highly variable from year to year and between populations, both in terms of the adult sex ratio and in the order of arrival of the sexes (examined fully in Chapters six and seven). Secondly, female smooth newts mate several times at the beginning of the season and then re-mate only occasionally during the rest of the breeding season (Hosie 1992, this chapter). Thirdly, male smooth newts have a dissociated breeding cycle (Crews 1987) and fourthly, research has shown that, in many species, male ejaculates may be more costly to produce than was once thought (Dewsbury 1982).

A number of experiments have demonstrated a reduction in the spermatophore supply of smooth newts, both over the duration of the season (Halliday 1976, Waights 1998) and within a single evening (Verrell 1986b). The number of sperms per spermatophore is also reduced in the course of a single night (Waights 1998). In an experiment which examined the responses of five males to females held in a strait-jacket, Halliday (1976) found that spermatophore production declined from an average of three to zero over the 40 days in which all individuals were tested daily. Males were tested to 'sexual exhaustion', determined either by the male fleeing from the female or after a minimum period of 120 seconds had elapsed since the previous deposition. In order to look at shorter term constraints on spermatophore production, Verrell (1986b), using the same method, tested males 5 minutes, 12 hours, 24 hours and 48 hours after initial deposition. A recovery period of 48 hours was necessary for the number of spermatophore depositions to return to the original maximum value, while after 12 hours, males deposited on average 66% fewer spermatophores. These findings formed the basis for the design of much of the subsequent experimental work on *Triturus vulgaris*.

The potential reproductive rate of smooth newts is thus influenced by the irregularity of female receptivity during the season and the limited sperm supply of males. This, together with the diverse patterns of arrival, leads to variation in the OSR throughout the season. As described in Chapter one, Verrell and McCabe (1988) suggested that, at the beginning of the season, when female demand for sperm is at its highest, male spermatophore supply may be temporarily depleted and a female-biased OSR may arise. Under these circumstances it would be expected that females would compete for males, while males would be discriminating in their choice of female. Female competition for males in *Triturus vulgaris* has indeed been observed at the beginning of the season (Waights 1996), while a male preference for larger females has been recorded in the urodele species *Notophthalmus viridescens*, *T. vulgaris* and *Desmognathus ochrophaeus* (Verrell 1985c; 1986a; 1989b).

Conversely, following the onset of ovulation, Verrell and McCabe (1988) proposed that female receptivity is reduced, the operational sex ratio becomes heavily male-biased and males are predicted to compete for choosy females. Although there is some evidence for female choice in smooth newts, this has not been as easy to demonstrate as might be expected on these theoretical grounds (section 4.4.2). Male-male competition, on the other hand, has been described in detail.

Amongst the urodeles, competition between males frequently takes the form of *sexual interference*, a strategy by which males disrupt the courtship of other males, seeking to enhance their own relative fitness by reducing the chances of insemination by rivals (Arnold 1976; Halliday 1998). Since (with the exception of two families; Hynobiidae and Cryptobranchidae), fertilisation is internal in urodeles but sperm is transferred externally via a spermatophore (Salthe 1967), the males are particularly vulnerable to this type of competition. Sexual interference may occur in a variety of ways, including placing one spermatophore on top of another (Arnold 1976), wrestling during amplexus (Verrell 1983) and female mimicry (Arnold 1976; Verrell 1984a); for review see Halliday (1998).

Sexual interference by female mimicry in smooth newts was first described by Verrell (1984a) following an experiment using triads (two males and one female). He observed interference in 59% of the triadic encounters, 82% of which involved female mimicry. As the courting male turns into creep, the interfering male inserts himself between the courter and the female. By touching the tail of the courter (mimicking female behaviour) the interferer elicits a deposition, then moves into creep himself, enticing the female to complete the spermatophore transfer phase and pick up his own spermatophore. Having felt the tail-touch from the rival male, the courter is unable to retaliate, since he is obliged to continue with the stereotyped actions of the spermatophore transfer phase. A single individual can adopt the role of both courter and interferer.

Females, however, do not always respond favourably to interference and have been observed to flee at the approach of the rival in both laboratory and field situations (Verrell 1984a; Massey 1988; 1988a; Faria 1995). This dislike of interference may be due to the action of the rival in obstructing courtship with a preferred partner (Verrell 1984a) since even females which attempt to pick up may be prevented from doing so (Chapter five). The extent to which females react negatively to interference is, however, dependent on the stage of courtship at which the interferer intrudes; the later the sequence within the encounter, the less likely the female is to flee (Verrell 1984a).

4.1.2 Variation in mating success and sexual selection

Darwin (1874) coined the term sexual selection to account for the evolution of characteristics which contribute to the reproductive success of an individual but which confer no advantage in terms of survival, '... his special weapons of offence, as well as his special means of defence, have been acquired through that form of selection which I have called sexual. This does not depend on any superiority in the general struggle for life, but on certain individuals of one sex, generally the male, being successful in conquering other males, and leaving a larger number of offspring to inherit their superiority than do the less successful males. There is another and more peaceful kind of contest, in which the males endeavour to excite or allure the female by various charms.' (Darwin 1874, p548, referring to mammals).

The evolution of sexual characteristics by sexual selection depends on *intra-sexual variation in mating success*. In the presence of such variation, sexual selection can operate to change the mean value of a phenotypic trait which covaries with mating success, the amount of variation directly influencing the intensity of selection (Wade and Arnold 1980; Arnold and Wade 1984). The evolutionary response to selection depends on the heritability of that trait (Lande and Arnold 1983).

The presence of variation in mating success thus indicates that the *opportunity for sexual selection* exists. Bateman (1948) was one of the first to investigate variation in mating success, using dominant marker genes to identify and quantify progeny from *Drosophila* matings. Whereas only 4% of females failed to produce any progeny, the same was true of 21% of males. Other examples of variation in mating success are readily found in the literature (Fincke 1982; Clutton-Brock 1983; Price 1984).

Linking variation in mating success to phenotypic traits and quantifying the result of selection on those traits is less straightforward (Harvey and Bradbury 1991). For example, since selection acts on the phenotype of the organism, it affects many characters simultaneously, several of which are likely to be highly correlated (Harvey and Bradbury 1991). In many species, phenotypic traits which correlate with mating success are also life history traits (such as body size) and therefore frequently under environmental control during part of the life cycle (Partridge and Halliday 1984; Price 1984; Halliday and Tejedo 1995).

Mating success is one component of *total reproductive success* which determines the number of surviving offspring per individual and which consists of several factors, including longevity, realised fecundity and offspring survival (Arnold and Wade 1984). Each component of reproductive success will typically be influenced by different factors and constrained by different selection pressures. In order for sexual selection to occur, mating success must make an important contribution to total reproductive success over the *lifetime* of the individual (Koenig and Albano 1986).

Variation in mating success is closely related to the operational sex ratio and the degree of competition for mates. Within most mating systems, a male-biased OSR will generate competition between males and variation in male mating success, while the reverse is true of a female-biased OSR. However there are exceptions; for example, a female bias may actually increase the variance in male mating success if few males remain able to monopolise females

(Partridge and Endler 1987). In general, males of polygynous species show greater variation in mating success than females of the same species and greater variation than males in monogamous species (Clutton-Brock 1983; McVey 1988).

The use of the artificial pond allowed me to identify individuals within the population and examine the variation in mating success among males and females.

Aim: ***to investigate the nature of competition between individuals and to quantify individual variation in mating success.***

Specific questions:

1. *How often does sexual interference occur and what are the consequences for spermatophore transfer?*

2. *What is the variation in mating success among males and females?*

4.2 Methods

Observation of the semi-natural population took place three to five times a week for approximately three hours in 1994 and 1995 (see Chapter three, section 3.2.2 for details). The artificial pond was scanned every 15 minutes and the behaviour of each individual within the pond and its position in relation to other newts was recorded. Between scans, observations focused on sexual behaviour. Courtship, likely to lead to spermatophore deposition, was given priority and all observations were timed and described, using a voice-activated tape-recorder.

4.3 Analysis and results

4.3.1 Sexual interference

Four types of sexual encounter, observed during focal sampling, were defined in Chapter three (section 3.3.2). In brief, these were OA: orientation with approach only; OD: orientation with display (no positive response by the female); RD: static and retreat display in which the female responded positively to courtship but no spermatophore deposition occurred and SP: courtship containing spermatophore deposition, with or without pick-up. In 1994, an additional

category (MM) was recorded in which males displayed to other males in the *absence* of females (Table 4.1).

Within the four main categories, five additional behaviour patterns which described interactions between individuals were defined and quantified (Table 4.1).

1. Scramble (sensu Sparreboom (1996)). Two or more males displayed to one female but either the rival male(s) or the female moved away before spermatophore deposition occurred.

2. Male-male interaction (M-m).
   A *courting* male approached or was approached by one or more other males. This often occurred as a result of scramble.

3. Male interference (M int.).
   During the spermatophore transfer phase, the interfering male moved between the courter and the female, enticing her into spermatophore transfer with himself. This resembled female mimicry as defined by Verrell (1984a) but did not always include tail-touch by the interferer.

4. Female-female interaction (F-f).
   A female receiving courtship was approached by another female.

5. Female interference (F int.).
   A female interfered during the spermatophore transfer phase, preventing insemination of the courting female and, in some cases, picking up the spermatophore herself (Waights 1996).

1994	**Total number**	**Single pair**	**Scramble**	**M-m**	**M int.**	**F-f**	**F int.**
OA	625	466 (74.5)	121 (19.5)	35 (5.5)	-	5 (1)	-
OD	537	379 (71)	13 (2.5)	43 (8)	-	3 (0.5)	-
RD	12	6 (50)	5 (42)	1 (8)	-	0	-
SP	39	17 (43.5)	6 (15.5)	3 (7.5)	16 (41)	3 (7.5)	0 (0)
MM	423						
1995							
OA	369	307 (83)	34 (9)	21 (5.5)	-	0	-
OD	579	450 (78)	82 (14)	63 (11)	-	1 (0.5)	-
RD	11	7 (63.5)	4 (36)	0	-	0	-
SP	30	10 (33.5)	6 (20)	1 (3)	15 (50)	0	0 (0)

Table 4.1. Interactions during different types of encounter in the semi-natural population. Number (%). M-m: interaction between males. M int: male interference. F-f: interaction between females. F int: female interference.

Interactions between females (F-f) were rarely observed and female interference as defined by Waights (1996) was never seen. On two occasions, a second female was observed attempting to move between a courting pair, but neither case resulted in successful

interference. Male-male interactions, on the other hand, occurred both during encounters with females (M-m) and often in the absence of females (MM). In fact, 25% of all sexual encounters took place between males only. In addition to their attempts to engage females in courtship, males clearly spent a considerable time chasing and displaying to other males.

During the orientation phase (OA and OD), the most frequently observed courtship (70 – 80%) involved a single pair of undisturbed newts. Courtships reaching static and retreat display (RD) however, contained an increasing amount of scramble. Scramble and male-male interaction usually resulted in the female swimming away or the males losing sight of the female. More than half the courtships reaching deposition contained interactions from rival individuals, male interference contributing the most (41% and 50% in 1994 and 1995 respectively). Interference followed a similar pattern to that described by Verrell (1984a) as female mimicry, but with some variations:-

1. The female touched the male's tail but missed the spermatophore, either because she turned away or because she approached the interferer (63% of sequences).
2. The female touched the male's tail and picked up despite interference (16% of sequences).
3. The female turned away or turned to follow the interferer just before tail-touch (10% of sequences). This is similar to 'Lure away' described by Sparreboom (1996) for *Cynops ensicauda popei.*
4. The remaining 11% of sequences in which the interfering male touched the courter's tail adhered to Verrell's definition of female mimicry.

In their field study, Verrell and McCabe (1988) found that the number of observations involving one male and one female (potential courtship encounters) decreased over the season, while those involving more than one male and one female (potential competitive encounters) increased. They interpreted this to mean that sexual interference was more prevalent later in the season when the OSR was highly male-biased. Using my data from scan sampling, the

number of sexual encounters (orientation only) per scan which involved a pair (dyad) or more than one male (triad) was obtained (Figure 4.1).

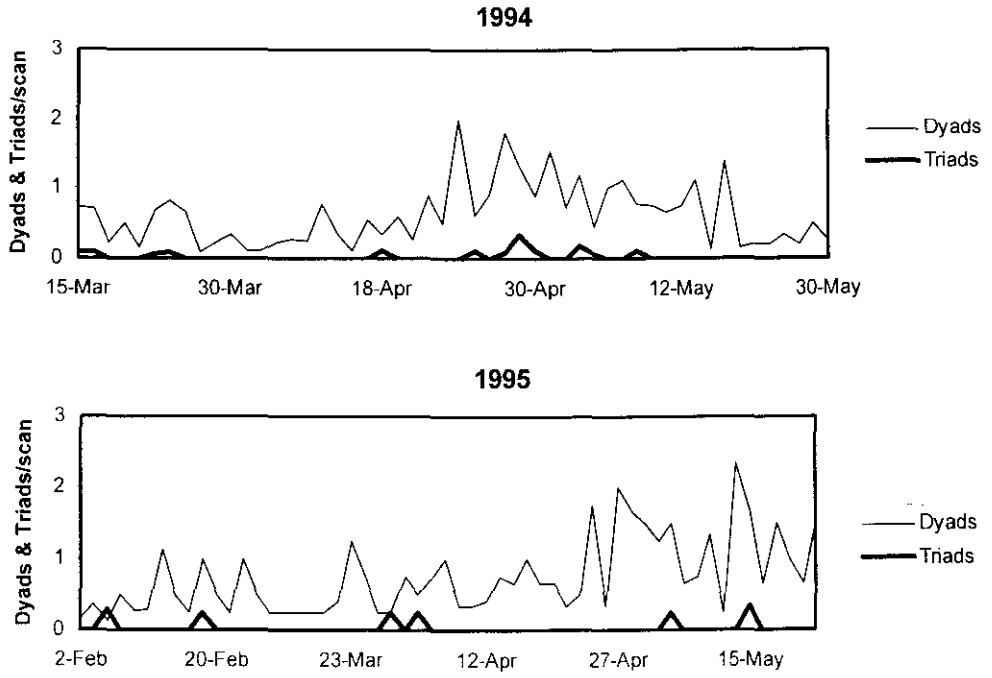


Figure 4.1. Number of dyads and triads per scan over the season.

The results do not corroborate those of Verrell and McCabe. The number of dyads increased in the second half of the season, coinciding with the general increase in male sexual activity associated with warmer temperatures (Figure 3.4 and Figure 3.5, Chapter three). Triads were much less frequently observed during scans and showed no obvious increase or decrease over the season.

How did sexual interference affect the mating success of courting and interfering males? In encounters containing interference in more than one sequence, it is necessary to define which male is the courter and which the interferer. I took the following approach. If, *following deposition* by the courting male, the interferer succeeded in enticing the female into courtship with himself, he was named the courter for the next sequence. The previous courter could then interfere. If the interferer drew the female away *prior to deposition* by the original courter, there was no change of sequence and he remained the interferer.

	1994		1995	
Total encounters (SP) with interference	16		15	
Courting male only deposited	10		10	
Interfering male only deposited	1		1	
Both males deposited	5		4	
	Depositions	**Pick-ups**	**Depositions**	**Pick-ups**
Total depositions and pick-ups in courtships containing interference	39	7	31	4
Courting male	31	7	25	3
Interfering male	8	0	6	1

Table 4.2. The effect of interference on the insemination success of courting and interfering males.

In the majority of cases, the courter managed to deposit spermatophores despite interference and, on several occasions, both males put down spermatophores (Table 4.2). It was rare for the interfering male only to deposit and insemination by the interfering male was particularly unlikely (one pick-up during the two years of observation in a total of 31 courtships containing interference). Table 4.3 compares spermatophore transfer in encounters with and without interference

	1994		1995	
Interference	**With**	**Without**	**With**	**Without**
Deposition	39	35	31	25
Pick-up	7	9	4	11
% Pick-up	17.9	25.7	12.9	44

Table 4.3. Spermatophore transfer with and without interference.

Although these data are not independent and cannot be tested statistically, there appears to be a reduction in pick-up success during encounters with interference.

4.3.2 Mating success

Where possible, the identity of individuals involved in courtships with deposition was recorded. Table 4.4 shows each courtship in which females could be recognised (not including new females) in 1994. The female is given in the left hand column with the identifying number of the courting male(s) below the appropriate date. A number in bold indicates that pick-up occurred during the courtship.

Female number	15 – 23 March	25 April	26 April	29 April	30 April	8 May
1						70
2	?, 3					
4	4				**20**	
7	7			7		
8	7		7			
11	?, ?					
12	9, 4, 7					
17	2	**10**				7
30	8					
60	9					

Table 4.4. Individual males and females in courtship with deposition, 1994. A number in bold indicates that pick-up occurred during the courtship. ?: identification of the male was uncertain.

This represents only 15 from the total of 39 courtships reaching the spermatophore transfer phase in 1994. Another 11 involved new females, leaving 13 with unidentified females (most of which occurred during the first week). In 1995, females were identified in all 30 courtships with deposition (Table 4.5).

Female number	3 – 13 Feb	22 Feb	21 Mar	8 Apr	12 Apr	25 Apr	1 May	5 May	10 May	19 May	26 May
2	**11**			**20**							
7	9	?	2,17					?			
8					**20**						
9	30						17, 4, 0			4,17,22 **0,80**	
10	**30, 3,** 60,17										
12	11,10, 9				?						
20	11,3							**22**, ?			
30											22
32						?					
4 toes									**22**,20		

Table 4.5. Individual males and females in courtship with deposition, 1995. A number in bold indicates that pick-up occurred during the courtship. ?: identification of the male was uncertain.

It must be stressed that this is an incomplete picture of courtship since sexual activity certainly occurred outside the hours of observation. It is likely that females which were evidently receptive but failed to pick up, continued to mate until pick-up was achieved.

Nevertheless, these data illustrate that both males and females mated with different individuals on a single night and over the season and that some individuals mated more frequently than others. The low frequency of pick-up is also clearly demonstrated.

Until now, I have expressed pick-up success as an overall percentage of spermatophore deposition without taking individual variation into account. Figure 4.2 illustrates the pitfalls of this approach.

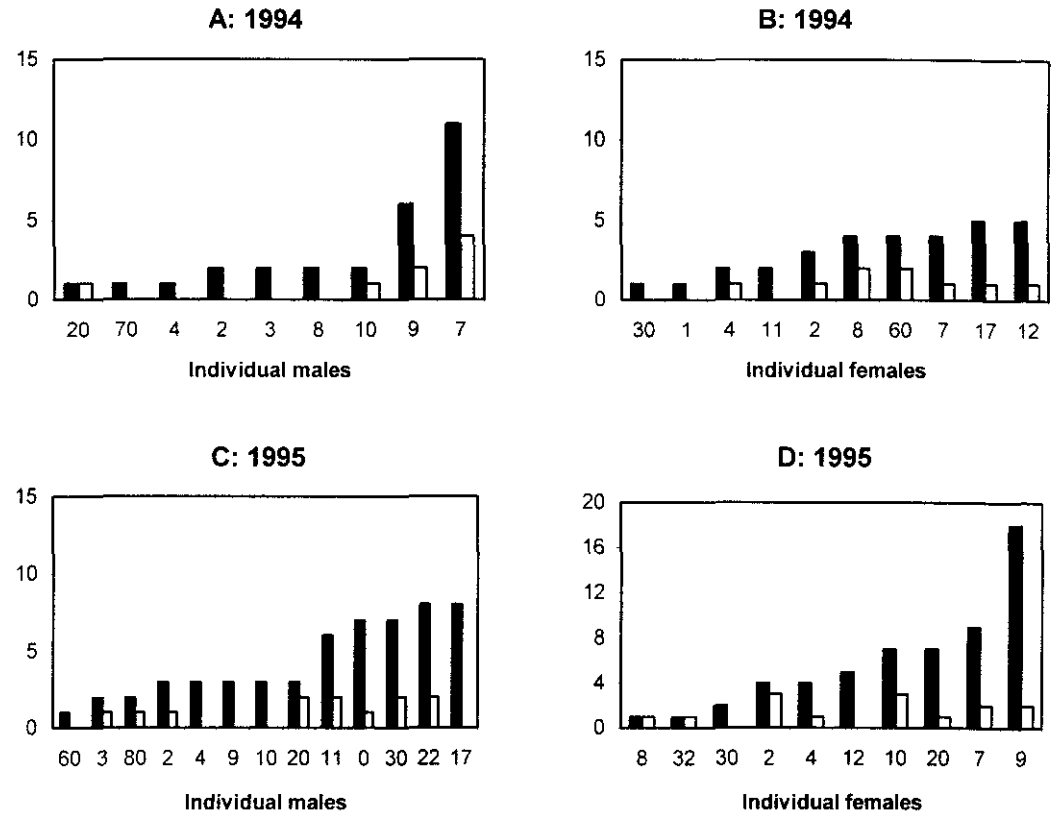


Figure 4.2. A and C: number of depositions by individual males and the number picked up over the season. B and D: number of depositions for individual females and the number picked up over the season. Black bars: deposition. White bars: pick-up. Individuals ordered according to number of depositions.

There was considerable individual variation in pick-up success. Although in 1994 males which deposited more also gained a higher number of inseminations, this pattern was not repeated in 1995. Male number 17, for example, deposited eight times but failed to inseminate any females while M20 achieved the maximum of two inseminations with only three depositions. The discrepancy between depositions elicited and picked up by females was even

greater. In 1995, F9 gained 18 depositions but only picked up two while F2 picked up three out of four spermatophores.

The variation in mating success between individuals represents the opportunity for sexual selection and can be measured by the standardised variance; $\sigma^2/\bar{x}^2$ (equivalent to the squared coefficient of variation) (Wade and Arnold 1980; Clutton-Brock 1983; Partridge 1988). Wade and Arnold (1980) considered mate number to be a more appropriate measure of mating success than copulatory success. In smooth newts, however, it is now known that polyspermy occurs during fertilisation (Waights 1998) and further research is needed to clarify the way in which the distribution of spermatophores between females affects mating success. The mean and standardised variation were therefore calculated for both the number of inseminations per individual and the number of mates per individual (Table 4.6). Mate number included only those mates with which insemination occurred. The percentage frequency distribution of mate number per individual is illustrated in Figure 4.3.

	1994			**1995**		
	$\bar{x}$	$\sigma^2/\bar{x}^2$	**n**	$\bar{x}$	$\sigma^2/\bar{x}^2$	**n**
Inseminations per male	0.88	2.35	9	0.92	0.87	13
Inseminations per female	0.9	0.67	10	1.4	0.59	10
Number of mates per male	0.77	1.97	9	0.76	0.88	13
Number of mates per female	0.8	0.63	10	1.2	0.43	10

Table 4.6. Mean and standardised variation in the number of inseminations and the number of mates per individual. n: number of individuals.

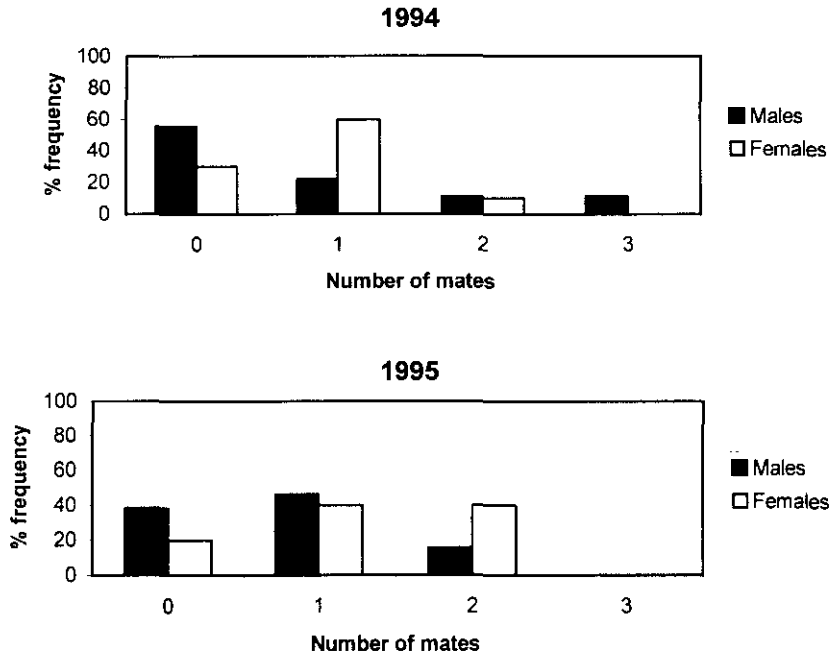


Figure 4.3. Percentage frequency distribution of the number of mates per individual, 1994 and 1995.

Insemination success and mate number per individual produced similar results (Table 4.6). Variation in mating success was greater among males than females but the difference was less marked in 1995. The range in mate number per male was surprisingly low with a maximum of three in 1994 (Figure 4.3).The difference in variation in mating success between the sexes is clarified by considering the number of individuals which failed to mate: 55.5% of males and 30% of females in 1994; 38.5% of males and 20% of females in 1995 (Figure 4.3). Although it is likely that all females would eventually have become inseminated, these results indicate that some males may never achieve insemination during a particular season.

In 1995, the identity of interfering males was recorded (Figure 4.4).

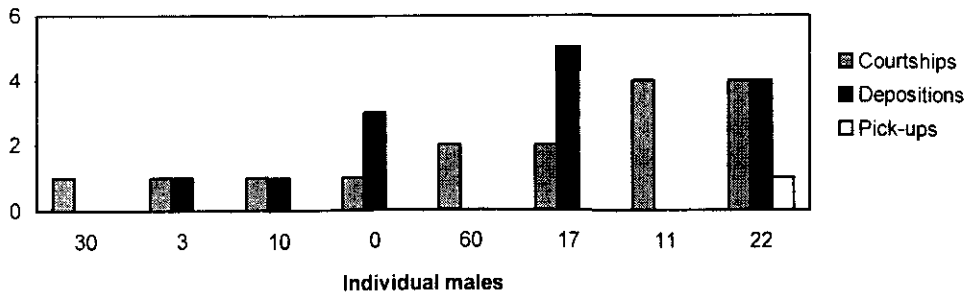


Figure 4.4. Interfering males: the number of courtships, depositions and pick-ups, 1995.

There was a complex relationship between the frequency of interference and the number of depositions or inseminations achieved as a result. For example, M22 interfered four times, deposited four times and attained one insemination, while M17 interfered twice, deposited five times but failed to inseminate either female. Noting again that this is a small sample size, it is interesting to compare Figure 4.2C with Figure 4.4 above. The males which deposited most spermatophores (more than three) were also those exhibiting sexual interference.

4.3.3 Biometrics

The crests of smooth newts have all the hallmarks of secondary sexual characteristics, developing only in males during the breeding season and displayed prominently during courtship (Halliday 1977a). Even Darwin (1874) referred specifically to the crest of male smooth newts, 'we may therefore conclude that the males have acquired their strongly-marked colours and ornamental appendages through sexual selection.' An association between crest height and mating success is needed to demonstrate the evolution of this character through sexual selection. Body size is another morphological characteristic often associated with mating success and sexual selection (Chapter five).

Weight and crest height vary across the season (Griffiths and Mylotte 1988) and were measured once a month during this study. Weight was used in preference to snout-vent length since it can be measured more accurately without resorting to anaesthesia. In 1995, the biometrics of the eight inseminating males were established for the nearest month to the date of insemination and characterised as above, near or below the mean value for that month (Table 4.7).

		10 Feb	6 March	12 April	8 May	Total
Crest height	**Above**	1		1	3	5
	Near	2				2
	Below		1			1
Weight	**Above**	1		1	2	4
	Near	1				1
	Below	1	1		1	3

Table 4.7. The number of inseminating males whose crest height or weight fell above, near or below the overall average at the time of insemination.

Despite the small sample size, these results suggest that during the second half of the season when crests were fully developed, all inseminating males had larger than average crests. There was no apparent relationship between weight and insemination success.

4.3.4 Summary of results from Chapter four

The results presented in this chapter were obtained during observation of the semi-natural population. Since the primary aim was to look at the effect of temperature on courtship behaviour over the season, many of the data are based on small sample sizes which cannot be statistically validated. Nevertheless, I believe that in view of the scarcity of data available on newt sexual behaviour outside the laboratory and the novel results provided by the identification of individuals, they represent a valid contribution.

Sexual interference

1. Most (70 – 80%) short bouts of courtship (OA and OD) involved an undisturbed pair of newts but sexual interference occurred in approximately half the encounters with deposition.

2. There was no variation in the amount of interference observed over the season.

3. Interfering males were rarely successful in inseminating the female but interference reduced the pick-up success of courting males.

4. Male-male interactions were frequent and usually occurred in the absence of females, whereas interactions between females and female interference were rarely observed.

Mating success

1. Variation in mating success was greater among males than females, both in terms of insemination success and number of mates, but the effect was more marked in 1994 than 1995.

2. There was some indication that a higher than average crest is characteristic of those males which successfully inseminate females, later in the season.

3. There was no correlation between the number of spermatophores elicited and the number picked up by females.

4.4 Discussion

4.4.1 Sexual interference

Sexual interference between males clearly forms an integral part of the courtship behaviour of smooth newts and affects the mating success of both males and females. Indeed, Halliday (1990) argued that sexual interference is an ancient behaviour pattern and that much of the courtship diversity in urodeles is the result of co-evolution between sexual interference and counteractive defence strategies. Sexual defence can be seen in extant species in a variety of forms (Arnold 1976; Green 1991b; Halliday 1998) including an increase in the duration of retreat display in order to draw the female away from the rival (Verrell 1984a).

In the present study, the incidence of interference increased as courting pairs reached the later stages of an encounter. One reason for this may be that females are less likely to flee from the interfering male as courtship progresses (Verrell 1984a). Males appeared to be attracted towards rivals engaged in retreat display and creep, and were probably responding both to the visual aspects of display and to pheromones released during the spermatophore transfer phase (Belvedere et al. 1988; Sparreboom and Ota 1995). Faria (1995) on the other hand, found that most interactions between male *T. boscai,* (14 of the 16 courtships observed in the wild) took place *before* the spermatophore transfer phase. Faria interpreted this as a type of sexual defence, the courter avoiding deposition in the presence of interfering males. Faria also described a behaviour by the interfering male which appeared to counter the tendency of females to flee. Instead of approaching directly, the rival adopted a 'waiting position' behind the courter, allowing their scents to mingle.

How do the results from this study compare with other reports of sexual interference in *Triturus* and closely related genera? One problem with answering this question is the varied

use of the term interference. Since spermatophore transfer is rarely seen in the wild, 'interference' has often been used in field studies to describe any interaction between males in the presence of a female, observed during all stages of courtship. I agree with Sparreboom and Ota (1995) that it would be more appropriate to refer to this as 'competition' to distinguish it from sexual interference which occurs only during the spermatophore transfer phase. Table 4.8 summarises field and laboratory data for interference in *Triturus* and the most closely related species from the family Salamandridae.

Site	Species	Description of competition/interference	Frequency of occurrence	Stage of courtship	Outcome	Author
Lab	*T. v. vulgaris*	Female mimicry	45 (59%) of triads (n = 78)	SP	Inseminations*: courter 27%, interferer 16%, both: 4%	Verrell 1984a
		Display by rival males	Occasionally observed	All phases	Courtship ends prematurely	
Semi-natural	*T. v. vulgaris*	Female mimicry and closely related forms (see text for details)	31 (45%) of encounters leading to deposition (n = 69)	SP	Inseminations*: courter 32.2%, interferer 3.2%, both 0%	Kauffmann, this thesis
		Display by rival males	433 (19.6%) of all encounters (n = 2202)	All phases	Female moves away, interfering male(s) give up or males display together	
Field	*T. v. vulgaris*	Female mimicry	(3) 5% of all encounters (n = 63)	SP	Female moves away, no inseminations	Verrell and McCabe 1988
		Display by rival males	21 (33.3%) of all encounters (n = 63)	All phases	Female moves away	
Field	*T. v. meridionalis*	Female mimicry	1 (2%) of all encounters (n = 57)	SP	Insemination by interfering male	Pavignano et al. 1993
		Display by rival males	31 (54%) of all encounters (n = 57)	All phases	Female moves away	
Field	*T. italicus*	Display by rival males	50 (67%) of all encounters (n = 74)	All phases	Courter spends more time with female	Giacoma and Crusco 1987
Field	*T. boscai*	Approach by rival males	154 (49%) of all encounters (n = 315)	All phases	Female usually moves away	Faria 1995
		Female mimicry	18 (5.7%) of all encounters (n = 315)	SP	No inseminations	

Site	Species	Description of competition/interference	Frequency of occurrence	Stage of courtship	Outcome	Author
Lab	*T. alpestris*	Display and creep by rival males	Not specified	All phases	Deposition by interfering male in 7.5% of encounters. Female usually moves away	Verrell 1988b
Field	*T. cristatus*	Display by rival males	20 (37%) of encounters lasting longer than 30 seconds (n = 54)	All phases	Female moves away, interfering male(s) give up or males display together	Hedlund 1990
Field	*T. cristatus* & *T. marmoratus*	Interruption by other males (unspecified)	60% of all encounters (n = 185)	All phases	Courtship ends prematurely	Zuiderwijk and Sparreboom 1986
		Female mimicry	A few occasions	SP	Rival may inseminate female	
Lab	*Notophthalmus viridescens*	Female mimicry	13 (24%) of encounters with amplexus (n = 55)	SP	Inseminations*: courter 69%, interferer 23%, both 7.6%	Verrell 1983
		Rival attempts to dislodge courter from amplexus	33 (60%) of encounters with amplexus (n = 55)	Amplexus	Rarely successful	
		Female mimicry	32 (70%) of hula encounters (n = 46)	SP	Inseminations*: courter 25%, interferer 40%, both 6.2%	
Field	*N. viridescens*	Female mimicry	48 (39%) of encounters with amplexus (n = 131)	SP	Inseminations*: courter 0%, interferer 6%, both 0%	Massey 1988
		Males clasped other males in amplexus	Frequently observed	Amplexus	Courter was induced to deposit on six occasions	
		Rival attempts to dislodge courter from amplexus	63 (48%) of encounters with amplexus (n = 131)	Amplexus	Never successful but increases time spent by courter in amplexus	

Site	Species	Description of competition/interference	Frequency of occurrence	Stage of courtship	Outcome	Author
Lab	*Cynops ensicauda*	Display by rival males	Not specified	Pursuit & fanning	Female usually moves away	Sparreboom 1996
		Female mimicry & Lure away	14 (70%) of triadic encounters (n = 20)	SP	Female moves away, no inseminations	
Field	*Cynops ensicauda*	Display by rival males	14 (14%) of encounters (n = 100)	Fanning	Not specified	Sparreboom and Ota 1995
		Female mimicry	11 (11%) of encounters (n = 100)	SP	Female moves away, no inseminations	
Lab	*Euproctus asper*	Signalling to rival males	Not specified	Before amplexus	Prevents interference from rival	Thiesmeier and Hornberg 1990
		Rival attempts to dislodge courter from amplexus.	Observed on a few occasions	Amplexus	Either fails results in all individuals moving away	
Lab	*Taricha torosa*	Rival pushes female aside during spermatophore transfer	Approximately 50% of encounters with amplexus (n = 37)	SP	2 inseminations by rival in 41 trials	Halliday and Hosie in prep.; Halliday and Tejedo 1995
Field	*Taricha torosa*	Many males grapple for the possession of one female	Not specified	Amplexus	Not specified	Arnold 1977

Table 4.8. Sexual interference in *Triturus* species and related species from the family Salamandridae. SP: spermatophore transfer phase. n: total number of encounters. Inseminations*: % of courtships containing interference in which the courting male, the interfering male or both achieved insemination.

Most authors have reported that sexual interference reduces the probability of insemination by the courter (exceptions being *N. viridescens* in amplexus, laboratory data (Verrell 1983) and *T. italicus* (Giacoma and Crusco 1987)). Following interference by female mimicry, insemination by either courter or interferer was rarely seen in the field. In one case (*N. viridescens*, Massey 1988) the interfering male achieved the same insemination rate as the courter but these data were based on just three inseminations out of 48 examples of interference observed over two years. Interfering males were also successful during female mimicry following hula display in *N. viridescens.* However, hula display, which leads to rapid spermatophore transfer in the laboratory when females are receptive, was not observed in the wild at all (Massey 1988). *Euproctus asper* exhibited an interesting behaviour in which males signalled to each other with tails raised in the air. It was thought that this might represent a strategy to prevent interference from rival males during amplexus (Thiesmeier and Hornberg 1990).

There are certain differences between interference observed in this study and the original description for smooth newts given by Verrell (1984a). Most notably, female mimicry, which was the commonest form of interference observed by Verrell, occurred in only 11% of courtships with interference in the semi-natural population. In the present study tail-touch was most commonly carried out by the female, after which she would approach the interferer, turn away altogether or pick up despite the interference.

Despite the differences between laboratory and field data, it can be seen from Table 4.8 that sexual competition usually results in the termination of courtship, depriving the courter of a mating opportunity and often increasing the cost of courtship by inducing sexual defence strategies. Sexual interference, on the other hand, can be viewed as an alternative mating strategy in which there is a small chance that the interferer will increase his own mating success through additional inseminations, but which almost always results in a reduction in the mating success of the rival male.

Since the discovery that smooth newts have a dissociated breeding cycle (Verrell et al. 1986), it has been assumed that the finite sperm supply imposes severe constraints on males. In the introduction I described two laboratory experiments (Halliday 1976; Verrell 1986b) which supported this concept. In this context, the reduction in pick-up success resulting from sexual interference has been described as a serious threat to the short- and long-term mating success of the courting male (Verrell 1984a).

However, these experiments were unable to take into account the apparent scarcity of opportunity for spermatophore deposition experienced by individuals in the wild (Table 3.4, Chapter three). For example, whereas Halliday (1976) recorded totals of from 27 to 75 (average 48) depositions per male over his 40 day experiment, the most successful males during my two years of observation deposited between 8 and 12 spermatophores over the entire season. Although I did not carry out observations during the early morning (when newts are also active) I never omitted more than one day's observation at a time and it is unlikely that this score would have more than doubled in reality. This suggests a maximum of 24 spermatophores per season for the most active males and less than ten for the average male. In a cross-sectional study, Waights (1998) found that males, taken directly from the wild and tested to sexual exhaustion, were still able to produce up to six spermatophores at the end of May. Over the season, it is appears that the opportunity to deposit arises too infrequently to cause depletion of the overall spermatophore supply.

It has been suggested that a temporary deficiency of spermatophores may arise at the beginning of the season if many receptive females arrive at once (Verrell 1986b). Verrell showed that males, tested to sexual exhaustion, deposited two to three spermatophores during their first courtship and needed 48 hours to replenish their supply. However, in the semi-natural population, the median number of depositions per male during a single encounter was only one (Table 3.9, Chapter three). There may be several explanations for this, including the presence of rival males, the complexity of the environment, temperature or deliberate

withholding of spermatophores by males for future matings. In *Notophthalmus viridescens*, males presented with four females deposited fewer spermatophores per partner than males presented with a single female (Verrell 1985b), although insemination of more than one female was rare. In this study, six males were observed to mate more than once in a single evening with a mean interval between matings of 55 minutes (Table 4.9). It appears that, contrary to previous supposition (Verrell and Halliday 1985a), spermatophore production in the short-term is also unlikely to be constrained by physiological capabilities.

Date	Male	1st Encounter		2nd Encounter		3rd Encounter	
		Time	Spa	Time	Spa	Time	Spa
15/3/94	8	20:30	1	21:45	1		
22/3/94	9	20:00	2	21:20	2		
10/2/95	9	21:15	1	21:20	2		
11/2/95	30	18:35	1	19:35	3	20:50	2
13/2/95	3	19:10	1	19:30	1		
26/5/95	22	20:30	1	21:30	1		

Table 4.9. The number of spermatophores deposited during separate encounters over a single evening, 1994 and 1995. Spa: number of depositions.

The cost of interference may thus lie more in the lost opportunity (the difficulty of finding another receptive female) than in the loss of spermatophores (see Sparreboom 1996). According to Dewsbury (1982), the search cost of encountering another female is an important factor influencing the distribution of male ejaculates. In the present study, males which interfered most frequently also deposited the most spermatophores. Despite their finite sperm supply, some males appear to follow a strategy of maximum deposition and interference in order to increase their chances of insemination. I suggest that it is the scarcity of opportunity for finding and inseminating receptive females in the wild which encourages them to do so.

Data from Verrell and McCabe (1988) indicated that competitive interactions between males were more likely to occur during the second half of the season. Although theoretically plausible, considering that receptive females are scarce and that the OSR is heavily male-biased at this time, this was not confirmed by results from the present study in which competitive interactions were equally prevalent throughout the season. The considerable

variation in local conditions between ponds, resulting from different arrival patterns of males and females (Chapter six), population densities and morphological characteristics may help to explain these discrepancies.

Frequent display between males has been described in *Triturus marmoratus* and *T. cristatus* (Zuiderwijk and Sparreboom 1986; Hedlund 1990), species in which males display to females in clusters (described as temporary leks). Hedlund suggested that such display may have evolved because females are attracted to male aggregations. Although there was no evidence to suggest that *T. vulgaris* formed temporary leks, display between males was also commonly observed, often in the absence of females (in contrast to observations by Verrell and McCabe 1988). In this species, interactions between males may be a means by which the quality of other males is assessed, although the most likely explanation is just one of mistaken identity (Giacoma and Crusco 1987).

In an experiment designed to investigate the occurrence of female interference across the season, Waights (1996) observed interference in approximately 43% of encounters with deposition prior to ovulation but none during post-ovulation encounters. Waights interpreted interference as a strategy used by highly motivated females to gain multiple matings at the beginning of the season when sperm may be a limiting resource. In the field, Faria (1995) reported a single observation of female interference in *Triturus boscai,* in which the female succeeded in disturbing a courting pair by pushing at the courter. In the present study, female interference sensu Waights (1996) was not observed. The intrusion into courtship by a second female occurred on two occasions, but in neither case was there disruption to the spermatophore transfer phase.

4.4.2 Mating success

Variation in mating success indicates that the opportunity for sexual selection exists. In the semi-natural population, despite multiple mating by females and despite a female-biased

sex ratio, the seasonal variation in insemination success and in number of mates was greater among males than among females and more males failed to mate than females (although these differences were much less marked in 1995). It is difficult to compare the opportunity for sexual selection between species because of the use of different indices (for example variation in male *breeding* success or variation in mating rate) which are often calculated across different sampling periods (Trail 1985; Clutton-Brock 1988). Nevertheless the standardised variances for number of mates per individual obtained in this study (males: 0.88 and 1.97, females: 0.43 and 0.63) are within the same range as those reported for other species. For example, variation in seasonal reproductive success in the monogamous kittiwake was 0.687 for both males and females (measured in terms of the number of young fledged) while that of the polygynous red deer was 2.23 for males and 2.04 for females (number of surviving calves. Clutton-Brock 1983). The seasonal standardised variance in number of mates was approximately 1.5 for male bullfrogs, similar to my estimate for *Triturus vulgaris*, while the variance in mates per female was considerably lower at 0.15 (Howard 1988).

If variation in mating success exists, it is necessary to ask what proportion of that variation is attributable to phenotype, to short term environmental variation, or to chance (Clutton-Brock 1988). Despite the small sample size (eight males) there was some indication of a positive relationship between crest height and insemination success during the second half of the season. A significant correlation between crest height and insemination success has been obtained under experimental conditions by Hosie (1992), Green (1991a) and Gabor and Halliday (1997) for *T. vulgaris,* Hedlund (1990) and Malacarne and Cortassa (1983) for *T. cristatus*. Both Hosie and Hedlund reported a stronger association during the second half of the season. Hedlund's data must be treated with caution since paired animals were left overnight and the number of spermatophores with the sperm cap removed was assumed to represent successful inseminations. This figure may be highly inaccurate, considering the frequency with which spermatophores fail to adhere to the cloaca. Gabor and Halliday (1997) found that

females did not discriminate between high- and low-crested males during their first courtship encounter but would subsequently only mate with high-crested males. There is also evidence that crest height in *Triturus* is associated both with body condition (following the terrestrial period) and with recent foraging success (Baker 1992b) and may thus represent an 'honest indicator' of male quality (Green 1991a). In addition, Waights (1998) demonstrated a correlation between maximum crest height and spermatophore production over the season, suggesting that high-crested males make an overall greater investment in reproductive resources.

Malacarne and Cortassa (1983) demonstrated that time spent in courtship is correlated with spermatophore transfer, body size and crest height in *T. cristatus*. However, larger crested males also have higher levels of prolactin and testosterone both of which influence the expression of courtship behaviour (for example display rate) as well as development of secondary sexual characteristics (see also Verrell et al. 1986). They concluded that both male persistence in increasing female receptivity and female choice for sexually mature males were important in determining non-random mating between individuals. Green (1991a) on the other hand, found no correlation between crest height and display rate in *T. cristatus*.

Houck et al. (1985) used analysis of variance to demonstrate that variation in mating success in the salamander *Desmognathus ochrophaeus* was based on intrinsic variation between individuals. The results showed variation in mating success of both sexes at the spermatophore transfer stage, but whereas in males this was due to among-male variance, in females it was attributed to random error. Females also varied considerably in the number of spermatophores that they elicited from males.

In *Triturus vulgaris* there may also be a significant short-term environmental component to the variation in mating success. The operational sex ratio varies during the season in

response to the arrival of new individuals (Chapter six) and changes in female receptivity. The effect this has on mating success is the subject of Chapter seven.

As mentioned in the introduction, in order for sexual selection to operate, mating success must make a significant contribution to total reproductive success. In species with polygamous females, *sperm competition* may play an important role in determining reproductive success in males (for a recent review of sperm competition in amphibians see Halliday 1998). Multiple mating in female smooth newts has been demonstrated in the laboratory (Verrell 1984b; Verrell 1984a; Hosie 1992) and in a more natural situation in this chapter. It occurs within a single encounter (as a result of interference), within a single night or after several days. A period of between 12 and 24 hours is required for all the sperm to migrate to the spermathecae (Sever et al. in press) and the relationship between different time periods following multiple insemination and male paternity is the subject of current research (Arano and Halliday unpubl. data). According to Sever et al. (in press) the tangled state in which sperm is stored and released on to the egg may represent an adaptation to reduce the effectiveness of rival sperm. Although further work is needed, it is clear that insemination of a female will not necessarily correlate with fertilisation.

In this study, variation in mating success was measured across a season but most calculations of variation in daily or seasonal reproductive success do not reflect variation in lifetime reproductive success (Clutton-Brock 1983). For example there may be a trade-off between the amount of energy invested in reproduction in one year and the ability of the animal to reproduce the next (Halliday 1992), or mating success may be correlated with longevity (Clutton-Brock 1983). It would be ideal to use a stable semi-natural population in which individuals can be identified to study long-term variation in reproductive success and the contribution made by mating success.

One of the most striking results to emerge from this study was the considerable variation among females in percentage pick-up; there was surprisingly little correlation between the number of spermatophores elicited by females and the number they picked up. From the male's point of view, increasing the number of depositions did not carry the certainty of increasing insemination success. This indicates that calculation of a mean percentage pick-up for a population may be very misleading in terms of individual success and raises the question again of why females pick up or miss spermatophores. According to Waights (1996), percentage pick-up by females during female interference is particularly high, implying that females *can* pick up when highly motivated. It is possible that some females choose to receive stimulation from male display without being motivated to pick up sperm; for example, such stimulation appears to be necessary for successful ovulation (Waights 1998). On the other hand, pick-up success is considerably lower in the field than in the laboratory, suggesting that the complexity of the physical environment (loss of spermatophores on the substrate, for example) and social environment (interference between males) also plays an important role in determining the success of pick-up (see also Chapter five).

4.4.3 Conclusions

How has the use of a semi-natural population contributed to the understanding of sexual behaviour in *Triturus*? The advantage of the semi-natural approach over field and laboratory studies is that active newts are always visible, can be identified and that sexual encounters can be readily observed while retaining a complex environment and a freely interacting population of newts.

Results from Chapters three and four confirmed that the scarcity of encounters with deposition, reported from the field (Table 3.4, Chapter three), is a genuine finding rather than an artefact of poor 'observability', and supported the idea that the intensity of sexual activity varies over the length of the breeding season (Verrell and McCabe 1988). The prevalence of sexual interference was corroborated (Verrell 1989a) and its importance in shaping the

outcome of sexual encounters, in particular pick-up success, was emphasised. However the hypothesis that males are handicapped by their finite spermatophore supply (Halliday 1987; Verrell 1989a) was not upheld since the number of depositions per encounter was small and the opportunity to deposit was infrequent. The identification of individuals was a novel approach. There was variation in mating success among males and females although the range in mate number per individual was not high. In particular, females varied in the number of depositions elicited and the percentage picked up.

There were also disadvantages to the semi-natural approach. Visibility in the water was very good in the artificial pond and the effect this may have had on communication between individuals is unknown. In this study the population was closed, obscuring any effect of immigration and emigration from the breeding site. In comparison with laboratory studies, the sample sizes were small. For example, despite having observed 30 courtships with deposition in 1995, I was reduced to a sample of just eight inseminating males from which to explore individual characteristics. Chapter five returns to an experimental approach to investigate two of the social issues that have arisen during the first four chapters: the influence of novel females on male courtship behaviour, and the outcome of interference between unevenly matched males.

Chapter five

Social factors: laboratory experiments

Experiment one: novel females

5.1 Introduction

In 1994, a female escaped from the artificial pond and a replacement female was introduced into the population. The result was a noticeable increase in male sexual activity. Subsequent introductions of both mated and unmated novel females produced similar responses, invariably resulting in the insemination of the new female. The present experiment was designed to investigate this phenomenon under more controlled conditions.

A preference for novel sexual partners has frequently been encountered in mammalian research and is termed the Coolidge effect (Dewsbury 1981). A reduction in mating activity occurs when one sex, typically the male, is faced with the same partner over an extended period, but mating activity is renewed with the introduction of a new mate. Most reports of this phenomenon come from studies of rats, hamsters, sheep and bulls but there have been few consistent results from any species (Dewsbury 1981). Whalen (1961) in Dewsbury (1981) compared the introduction of novel mated and novel unmated female rats and found that males copulated more frequently with unmated females. They suggested that this may have been due to resistance from mated females suffering from vaginal irritation. Krames and Mastromatteo (1973) observed that male rats prefer the odour of their original partner before ejaculation but that of a novel female after ejaculation.

Donovan and Verrell (1991) studied the effect of long-term partner familiarity in the salamander *Desmognathus ochrophaeus*. Males inseminate a maximum of one female per night and both sexes require a recovery period of about three days to maintain insemination rates over the extended breeding season. After approximately five encounters, the probability of successful courtship between familiar pairs of salamanders was significantly lower than the probability of insemination if the familiar female was replaced by a novel female. The reproductive state of the females was not specified. Although declining to give a functional

explanation, Donovan and Verrell pointed out the similarity between this and the Coolidge effect.

Aim: ***To investigate the effect of novelty on male sexual activity.***

Experimental hypotheses:

1. *The introduction of a novel mated or unmated female to an established group of newts causes an increase in male sexual activity.*
2. *Most of the increase in sexual activity is directed towards the novel female.*
3. *The probability of insemination of the new female is greater than that of a familiar female.*

5.2 Methods

Six tanks were set up with gravel, hiding places (bricks and broken flower pots) and egg-laying sites (plastic weed). The tanks were divided into two groups of three (ABC, DEF) since three was the maximum number of tanks which could be observed with accuracy during a single session. Four males and four females were placed in each tank in such a way that a similar range of body sizes was present in each tank. A period of three days was allowed for the newts to settle and to become familiarised with each other. Courtship during the first day of the settling period was observed and recorded using the following scores:-

1.	Males	Number of 'move-to-the-front' (MF), whips, fan bouts and spermatophore depositions.
2.	Females	Number of spermatophores picked up or missed.

Following the settling period, the novel females were introduced systematically, each set of three tanks being observed on alternate days. One female was removed from each tank several hours before the trial in order to maintain group size. At the start of the trial, a novel unmated female was placed in the first tank, a novel mated female in the second and in the third tank, the same female was replaced to act as the control. A counterbalanced design was used to avoid the problem of order effects (Table 5.1).

Day	Tank A	Tank B	Tank C	Tank D	Tank E	Tank F
1	Settle	Settle	Settle			
2				Settle	Settle	Settle
4	UM	M	C			
5				UM	C	M
6	M	C	UM			
7				C	M	UM
8	C	UM	M			
9				M	UM	C
10	UM	M	C			
11				UM	C	M
12	M	C	UM			
13				C	M	UM
14	C	UM	M			
15				M	UM	C
Day	**Tank G**	**Tank H**	**Tank I**			
20	Settle	Settle	Settle			
23	M	UM	C			
25	C	M	UM			
27	UM	C	M			
29	M	UM	C			
31	C	M	UM			
33	UM	C	M			

Table 5.1. Details of the procedure for the novel female experiment. UM: unmated novel. M: mated novel. C: control.

As a result, each group experienced all three conditions twice in different orders. Since groups were tested on alternate days, the animals had 48 hours to become familiarised with the new female before re-testing and males had sufficient time to replenish their spermatophore supply (Verrell 1986b).

The same elements of courtship were recorded as during the settling period but display directed towards new females was scored separately. In addition, the individuals involved in spermatophore deposition and sexual interference were identified. Observation sessions lasted 2.5 hours. Mated females were taken from among those females which had mated previously during the experiment and were chosen to ensure an even distribution of sizes across the six

groups. Females were never placed with the same group of males more than once. Another set of trials was carried out with three more tanks (GHI) bringing the sample size to nine.

Novel females (both unmated and mated) were housed under the same conditions as the experimental animals (but with more individuals to a tank). Temperature was maintained between 13°C and 15.5°C. The light/dark cycle was manipulated so that 'dusk' occurred at 15:00 hours when the observation session began. The number of light/dark hours was adjusted on a weekly basis to match London lighting-up times and varied from 10/14 hours to 8/16 hours. The experiment took place between 3 April and 5 May 1995.

5.3 Analysis and results

For the purpose of analysis, male display was divided into two categories: a) move-to-the-front and fan and b) whip. Move-to-the-front and fan (MF + fan) were combined because they are often associated during courtship, move-to-the-front usually being followed immediately by fanning (although the male may also fan at other times). In general, this part of the display represents male persistence in attracting the attention of the female. Once the female is stationary, the male is able to perform a series of whips which lead to the spermatophore transfer phase. Whips therefore indicate that a female is responsive to a male.

During analysis, I detected a small problem with the experimental design: whereas control females were removed and returned to the control tank after a period of 24 hours, unfamiliar mated females had been housed in isolation from males for a number of days. This was due to the need to match females for size, both across conditions and within each tank. Time spent in the absence of males ranged from less than a day (identical to control) to seven days but with an average of 2.6 days. This created a potential confounding factor since females may have behaved differently with increased time away from males. I therefore carried out Spearman rank-order correlations to see if there was a relationship between the amount of display

directed at the novel mated female and the length of time spent by that female in the absence of males (Statistics box 5.1, Figure 5.1).

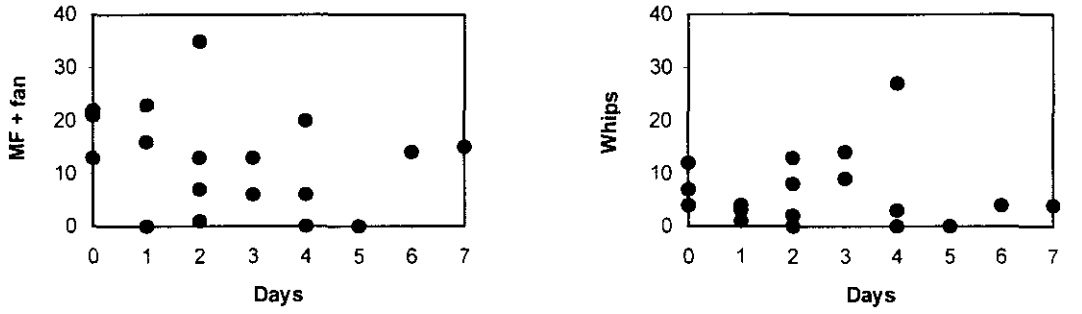


Figure 5.1. The total number of MF + fan and whips directed towards novel mated females in relation to the number of days they spent isolated from males.

The number of days spent without males had no effect on courtship behaviour and it was therefore thought reasonable to continue with analysis of the main experiment. Tests are two-tailed unless otherwise indicated.

The analysis was based on a within-subjects design since each tank was tested under each condition, the 'subject' being the group of four males. As an inevitable consequence of the need to establish familiarity, it was not possible for each condition to contain an independent group of females. For example, the control group contained the same individuals as those encountered under the previous condition (unless the control condition was first). Since the experiment was counterbalanced, I avoided any problems of consistent bias arising from this.

Statistics box 5.1.

Spearman rank-order correlation to examine the association between the amount of display directed at the novel mated female and time spent by the female in the absence of males.

MF + fan: $r_s = 0.01$, $n = 18$, $p > 0.5$. Whip: $r_s = 0.02$, $n = 18$, $p > 0.5$.

Throughout this analysis, UM is used to refer to the tanks containing the novel unmated female, M to tanks containing the novel mated female and C to the control tank.

5.3.1 Male sexual activity

The first aim of the experiment was to find out if the addition of a novel female increased male sexual activity. Friedman, non-parametric, two-way analysis of variance by ranks was carried out to test for differences in male display score between the three conditions (a non-parametric test being chosen because data were neither normally distributed nor homoscedastic). Scores from the two trials under each condition were totalled.

There was a significant difference in the amount of male display between the three conditions for both scores (Statistics box 5.2A). Multiple comparisons revealed a gradient in display score from C to M to UM and, while the two extremes (UM and C) were significantly different from each other, the central condition (M) was not sufficiently different from either of the other two to be statistically significant (Statistics box 5.2B). The trend of increasing male activity in the direction C to M to UM was more marked for MF + fan than for whips (Figure 5.2).

Statistics box 5.2

A. Friedman two-way analysis of variance to compare the amount of male display between unmated, mated and control tanks. MF + fan: $Fr = 6.84$, $n = 9$, $df = 2$, $\mathbf{p < 0.05}$. Whip: $Fr = 14.89$, $n = 9$, $df = 2$, $\mathbf{p < 0.001}$.

B. Multiple comparisons (Siegel and Castellan 1988, p 180) between display scores in unmated, mated and control tanks. Critical difference for $\alpha 0.025$ (one-tailed) = 11.19. The alpha level was reduced (Bonferroni's adjustment) because each score was used twice (Jaeger and Halliday in prep.). NS: not significant.

MF + fan. UM vs C: rank difference = 12, $\mathbf{p < 0.025}$. UM vs M: rank difference = 6, NS. M vs C: rank difference = 6, NS.

Whip. UM vs C: rank difference = 16, $\mathbf{p < 0.025}$. UM vs M: rank difference = 11, NS. M vs C: rank difference = 5, NS.

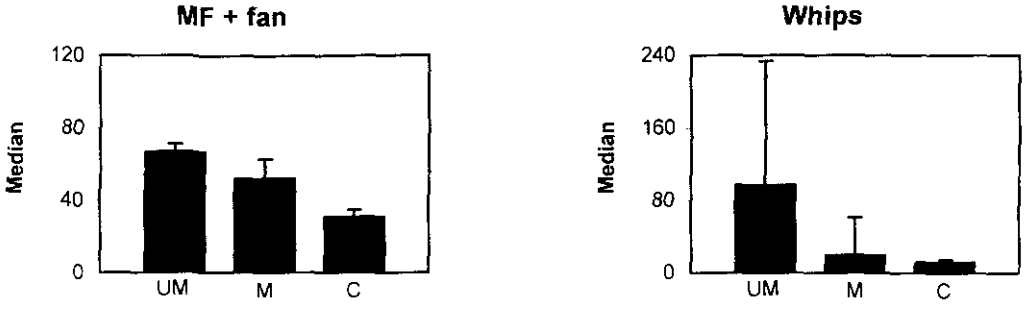


Figure 5.2. The median number of MF + fan and whips in the unmated, mated and control conditions. n (number of tanks): 9. 'Error bars' are used to indicate the 75th percentile. Note the different scales due to the large amount of variation in UM whip score.

The effect of novel females was also examined by comparing the amount of spermatophore deposition and pick-up. Unfortunately, the rare occurrence of spermatophore transfer presented problems with statistical analysis due to frequent zero scores. For example, neither the mean or median gave meaningful summaries of the data. Instead, total numbers of deposition and pick-up were used for comparison between conditions with the number of tanks in which deposition or pick-up occurred shown above each bar (Figure 5.3).

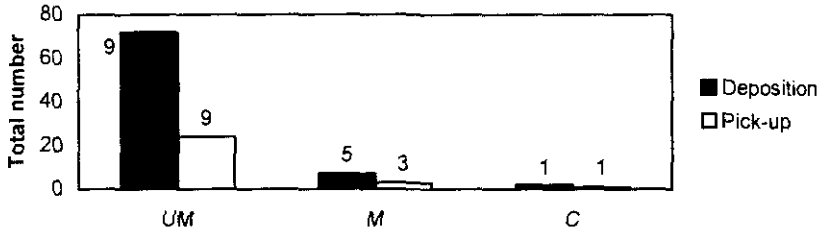


Figure 5.3. Total deposition and pick-up in the three conditions. The number by each bar refers to the number of tanks contributing to the total.

It was not thought necessary to carry out a multiple test since the amount of deposition and pick-up in UM was clearly different from both M and C. However, the sign test showed that

the slight difference between M and C was not statistically significant (Statistics box 5.3).

5.3.2 Novel versus familiar females

Given that, within a tank, there was an overall increase in male sexual activity as a result of the addition of a novel female, the next question is: what proportion of this activity was directed at the unfamiliar as opposed to familiar females?

Male display directed at the novel female was scored separately in the unmated and mated tanks. This was compared with the mean score for the familiar females (total score/3) using the Wilcoxon signed ranks test. A significant effect is apparent in both conditions, but this is greater in the case of unmated females (Statistics box 5.4A, Figure 5.4). Once again, within the mated condition, the effect is stronger for MF + fan than for whips.

Statistics box 5.3

Sign test (Siegel and Castellan 1988) to compare deposition and pick-up between mated and control conditions. One-tailed tests were used because deposition and pick-up were expected to be higher in the mated tanks. Sample sizes were reduced to six and four because this was the number of tanks in which deposition and pick-up occurred.

Deposition: $x = 5$, $n = 6$, p (one-tailed) = 0.109. Pick-up: $x = 3$, $n = 4$, p (one-tailed) = 0.312.

Statistics box 5.4

A. Wilcoxon signed ranks test to compare the amount of display directed at the novel female with the mean score for familiar females.

UM. Whip: $\mathbf{p = 0.004}$. MF + fan: $\mathbf{p = 0.004}$.

M. Whip: $\mathbf{p < 0.02}$. MF + fan: $\mathbf{p < 0.008}$.

B. Sign tests to compare deposition and pick-up for novel and familiar females. One-tailed tests were used since novel females were predicted to gain higher scores.

UM. Deposition: $x = 9$, $n = 9$, $\mathbf{p = 0.002}$. Pick-up: $x = 8$, $n = 8$, $\mathbf{p = 0.004}$.

M. Deposition: $x = 4$, $n = 4$, $p = 0.062$. Pick-up, sample size (3) is too small to use.

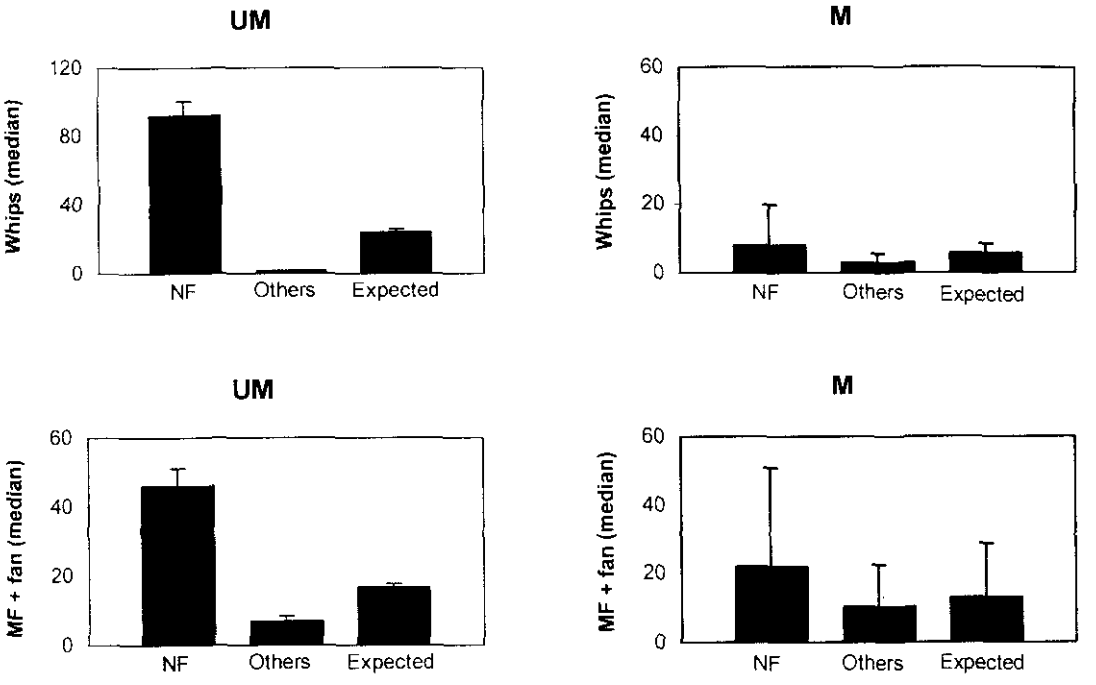


Figure 5.4. The median number of MF + fan and whips directed at the novel versus familiar females within UM and M tanks. n (number of tanks): 9. 'Error bars' are used to indicate the 75th percentile. NF: new female. Others: mean display directed towards the three familiar females. Expected: the amount of display which would have been received by each female if display was apportioned equally (total display/4). Note that the scale for UM whips is different.

Total scores of deposition and pick-up for novel and familiar females are shown in Figure 5.5. In both cases, novel females acquired more spermatophores than their familiar counterparts but this was only significant in the unmated tanks (Statistics box 5.4B).

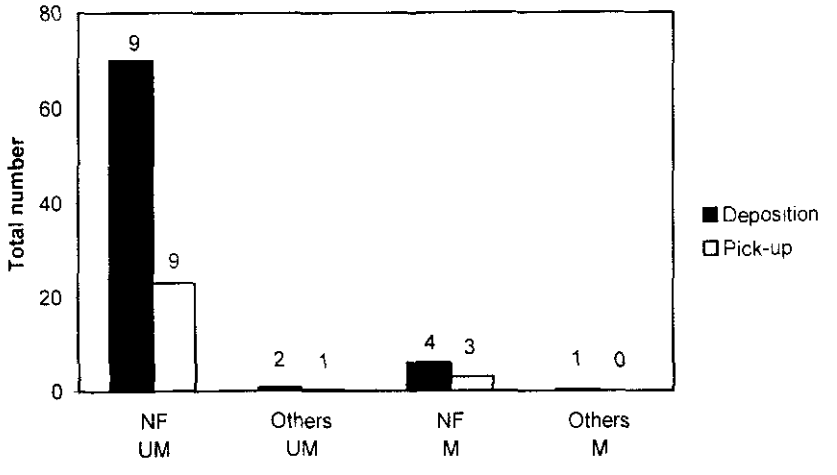


Figure 5.5. Total deposition and pick-up for novel versus familiar females in the unmated and mated conditions.

5.3.3 Summary of results from experiment one

1. The introduction of both unmated and mated novel females resulted in an increase in male sexual activity but this was only significant for unmated females. *Reject null hypothesis one that novel females have no effect on male sexual activity.*

2. A significantly greater proportion of male display was directed towards novel females (both unmated and mated) than familiar females. For mated females, the effect was more marked in MF + fan than whips. *Reject null hypothesis two that there is no difference in the amount of display directed at the novel or familiar females.*

3. Novel unmated females were inseminated more frequently than novel mated or control females. In the case of the unmated female, *reject null hypothesis three that the probability of insemination of novel and familiar females is the same.*

4. Overall, the effect of unmated novel females on male sexual activity was much more marked than that of mated females.

5.4 Discussion

This experiment demonstrated a marked increase in male display activity following the introduction of a novel *unmated* female. Significantly more display was directed towards these females than to the familiar females, indicating the ability of males to distinguish between them. The insemination rates of novel unmated females were high in comparison with familiar females, illustrating their receptivity to courtship.

Recognition of unmated females by male smooth newts may be based on differences in female behaviour or on visual or olfactory cues. Proceptive behaviour by females at the beginning of the breeding season has been demonstrated by Hosie (1992) and Waights (1996) and was discussed in Chapter three. In an experiment in which females were manipulated to approach males at a relatively fast and slow rate, males tended to deposit more spermatophores

for the first female encountered (Halliday unpubl. data). The difference, however, was much greater if the 'fast' female was presented first, implying that males were influenced by the degree of female proceptivity. In the present study, the increase in male display towards unmated females was predominantly in the number of whips rather than MF + fan. Whereas males had no need to persuade the receptive females to engage in courtship (MF + fan), positive female response enabled males to reach retreat display (mostly whips) and spermatophore transfer.

It is also possible that unmated female smooth newts signal receptivity by visual or olfactory cues. Males, for example, spend a considerable time sniffing females at the start of courtship, especially around the cloaca (Marquenie 1950; Halliday 1972). The ability of males to detect the unmated status of females in the Japanese newt, *Cynops ensicauda popei* was reported by Sparreboom and Ota (1995) who observed 'a noticeable increase in male activity ... if a new and probably unmated female entered the pond'. The two inseminations observed during their field study occurred within 10 minutes and one hour of the females entering the pond. They suggested that females were conspicuous by their silvery body colour, the result of air bubbles trapped on the dry skin. However, this explanation is unlikely to apply to smooth newts which become fully aquatic before beginning to court.

Both visual and olfactory cues have been put forward to explain the ability of male *Notophthalmus viridescens*, *Desmognathus ochrophaeus*, and *Triturus vulgaris* to discriminate between large and small females (Verrell 1985c; 1986a; 1989b), although visual cues had a stronger effect in *T. vulgaris*. Verrell proposed vitellogenin as a possible olfactory candidate since it is circulated in proportion to the number of ova available for fertilisation (Verrell 1985c). However, quantitative comparison of olfactory substances may not be viable in the field (Rowland et al. 1990). For example, a cluster of small females may be more attractive to a male than a single large female. Females are seldom encountered at equal distances and the

dilution of odour would also be highly variable. They suggested that olfactory gradients were more likely to be used in the location rather than the selection of mates. This is supported by data on *T. vulgaris* which shows that olfactory rather than visual cues are used by males for the long range detection of females (Cogãlniceanu 1992; 1994). Unmated female smooth newts may nevertheless be distinguished by a qualitative difference in odour since they have probably undergone the hormonal changes associated with the aquatic phase more recently than mated females. The most likely explanation is that both olfactory and visual cues play a part in the recognition of unmated females.

The overall increase in male activity in response to *mated* unfamiliar females was not significant. Nevertheless, within the 'mated female' tanks, a significantly higher proportion of display was directed at the novel female. This was particularly strong in the case of MF + fan. It appears that despite the lack of consistent response, males were more persistent in trying to stimulate the novel mated females than familiar females. The poor success rate is indicated by the low number of whips and depositions. In this experiment, females were not affected by the number of days spent in isolation from males, although Green (1991a) reported that females kept for longer in stock tanks were less likely to pick up spermatophores. No explanation was given to account for this.

It is more difficult to postulate the mechanisms by which males recognised mated novel females. Since they were only marginally more responsive than familiar females, a difference in behaviour is not an appropriate explanation. It also seems unlikely that there is a particular odour 'badge' which would distinguish them from familiar mated females. Nevertheless Hosie (1992) demonstrated that, on consecutive days, females were more likely to mate with a different male than with a previously encountered male, suggesting that females can either distinguish between familiar and unfamiliar males or remember individual mating partners.

In an experiment which investigated individual recognition, Wass and Colgen (1994) showed that male sticklebacks (*Gasterosteus aculeatus*) were able to distinguish between two familiar rivals using visual cues alone. Jaeger (1986) in Donovan (1991) also found evidence of such an ability in a number of terrestrial salamanders. However, in territorial species such as these, the distinction between familiar and unfamiliar individuals is likely to be enhanced through repeated interactions with local rivals.

What are the possible advantages for male smooth newts in mating with an unmated, mated or unfamiliar individual? In the present experiment, the effects of novelty cannot be separated from reproductive state since unmated females were always unfamiliar. For mature females, this is also likely to be the case in the wild. In species which mate multiply, the advantages of courting unmated or mated females depends, to a large extent, on the outcome of sperm competition.

In many species in which there is first male precedence, males prefer to mate with unmated females. Examples are bowl and doily spiders (*Frontinella pyramitela*) in which female reproductive condition is assessed during courtship and pseudocopulation (Suter 1990), and funnel web spiders (*Aglenopsis aperta*) in which chemical cues emitted by females are attached to the web (Reichert and Singer 1995). Males of species with last-male precedence in sperm competition, on the other hand, should prefer mated females (Tokarz 1995). As mentioned in Chapter four, the details of sperm competition in *Triturus* are not yet known, although last male precedence is considered most likely (Rafinski and Pecio in Halliday 1998). However, in a species without territorial defence or the ability to mate-guard, attempting to be the last to mate with a female is a risky strategy. Alternative and more likely strategies for smooth newts are to mate at every opportunity and to interfere with other courtships (Chapter four). Under these circumstances it is advantageous to court unmated females since they clearly offer a greater chance of insemination than mated females.

There are also theoretical grounds for choosing an unfamiliar female, regardless of her reproductive state. Males with limited sperm supplies may enhance the genetic variability of their offspring by mating with as many different individuals as possible. If male smooth newt preference for unfamiliar females is associated with producing genetically diverse offspring, males should limit the number of spermatophores deposited for any one female, in particular at the start of the season. As discussed in Chapter four (section 4.4.1), Verrell (1985b) found that male *Notophthalmus viridescens* deposited fewer spermatophores per partner when four females were available than when a single female was present. Males were thus capable of altering their behaviour in relation to the number of available females. In addition, when four females were available, males preferred to mate with a different partner, following their first encounter.

There are two reports of male territorial lizards exhibiting a preference for unfamiliar females (*Holbrookia propinqua*, Cooper 1985; *Anolis sagrei*, Tokarz 1992; review in Tokarz 1995). Cooper suggested two ways in which a territorial male may benefit from this preference. The first was that the unfamiliar female may take up residency, allowing the male to gain a mate without incurring any search costs. The second was that the male may increase his reproductive success by mating with a female which may not be available in the future. However, neither of these explanations apply to the mating system of the smooth newt.

In species in which female receptivity varies throughout the breeding season, selection should favour the ability of males to test the receptivity of females (Thornhill and Alcock 1983). In view of the energetic cost of the elaborate courtship display of male smooth newts (Chapter two), it would evidently be advantageous for males to avoid wasting both time and energy in courting unreceptive females. At the beginning of the season, the ability of males to recognise unmated females is vital in order to gain rapid access to receptive females before they are detected by rival males. During the second part of the season, recognition of receptive

females is important when females only re-mate periodically. In smooth newts, the ability to distinguish between familiar and unfamiliar mated females may help to achieve this since there is a greater chance of an unfamiliar female being receptive than one which has already been courted unsuccessfully.

I suggest that the marked preference of male smooth newts for *unmated* females reflects the likelihood of successful courtship, while the increase in interest for *mated novel* females is due to the greater chance that an unknown female will be willing to re-mate. Both these hypotheses assume that male smooth newts are able to assess reproductive state and distinguish between unfamiliar and familiar individuals.

The ability of males to recognise receptive females has been demonstrated in other species (such as pygmy marmosets, Converse et al. 1995). Sailfin mollies (*Poecilia latipinna*) have a similar mating system to that of the smooth newt; population densities are typically high and while males are sexually active most of the time, females are only receptive as virgins and after parturition when new ova are released. Farr and Travis (1986) found that males were able to recognise females in the fertile part of their cycle and displayed considerably more towards virgin and receptive females.

Fertility/receptivity advertisement by females is clearly of benefit to males but does it benefit the female? Rowland et al. (1990) suggested that females improve their chances of obtaining a high quality mate as a result of increased male-male competition. Female sticklebacks (*Gasterosteus aculeatus*) indicate their readiness to mate by producing a temporary dark, mottled pattern on the back and upper flanks and males prefer females with this colouration (Rowland et al. 1990). It is debatable whether female smooth newts gain from increased male-male competition in this way. Although the increase in display rate of smooth newts in the presence of rivals (Verrell 1984a) may theoretically enable females to mate with

the most attractive males (Farr and Travis 1986), the outcome of sexual interference appears to be highly unpredictable (Chapter four and experiment two of this chapter).

The ability of male *T. vulgaris* to distinguish between familiar and unfamiliar females in the wild may be dependent on either low population density or the distribution of animals within the breeding pond (both spatial and temporal) preventing complete mixing of the population. Newts breed in water bodies of all shapes and sizes, ranging from large ponds to linear ditches. The latter could provide small pockets within which individuals become familiarised with each other and between which there is a certain amount of movement. Using mark-recapture, Dolmen (1981) traced the movements of *T. vulgaris* along drainage ditches during the breeding season and found that most individuals moved about 35m (maximum 120m) and that females travelled more extensively than males. In this way, unfamiliar females would pass through the vicinity of males. Where the density of water bodies is high, newts may travel between ponds or ditches during the breeding season as reported for *T. vulgaris* (Dolmen 1981; Griffiths 1984) and *T. alpestris* (H. Faber unpubl. data).

Experiment two: sexual interference

5.5 Introduction

Body size is often considered an important determinant of reproductive success. In females, large size is frequently associated with greater fecundity, and male preference for large females has been demonstrated in many species (for example wood frog, Berven 1981; sticklebacks, Sargent et al. 1986; smooth newt, Verrell 1986a).

Male body size is commonly associated with the ability to obtain and defend resources essential to reproduction, such as territories, nest sites or females themselves. For example, mating success is higher in large male bugs (*Margus obscurator*) which are able to prevent females from fleeing during copulation and control access to the sole food source (McLain et al. 1993). Size may also be associated with provision of short term benefits during the

reproductive process; for example, large male dung beetles (*Onthophagus binodis*) assist in finding food and guarding burrows, while small males do not (Cook 1990).

Positive correlations between mating success and body size have been found in several species of amphibian (review in Halliday and Tejedo 1995) although there is often a lack of within-species consistency between years and populations. In *Bufo bufo*, large males are more successful at dislodging rivals from amplexus (Davies and Halliday 1979) but the extent of this advantage varies from year to year (Halliday unpubl. data). Houck (1988) reported that small male salamanders *Desmognathus ochrophaeus* never achieve insemination in the presence of a large conspecific, due to aggressive behaviour by the larger males. However, the benefit of being large in competitive situations does not necessarily involve direct physical contact since a large size in itself may be sufficient to inhibit aggressive behaviour by smaller males (Huntingford and Turner 1987).

In the absence of direct competition between males or immediate benefits during reproduction, a female preference for larger males can be understood in functional terms if body size (or a correlate such as age, vigour, state of nutrition, status and disease resistance. Halliday 1992) is positively associated with either potential fecundity or genetic quality. A proximate explanation for the preference may be a positive association between size and the strength of the stimulus provided by the male (Rowland 1989).

In *Triturus vulgaris*, competition between males for mates takes the form of non-aggressive interference and large size confers no advantage in terms of physical combat. In addition, the role of courter and interferer during interference can be assumed by any individual (Halliday 1998). Most experiments investigating sexual interference in urodeles (Chapter four) have used males matched for size and other attributes (Verrell 1983; 1984a; Sparreboom 1996) and it has often been assumed that male morphological characteristics have no bearing on the outcome of interference (Halliday and Verrell 1986; Green 1991a).

However, in trials using two males and one female, Reques and Halliday (submitted), found that small male *Triturus pygmaeus* spent less time courting the female than larger males. Deposition (in 3/22 trials) was always by the larger male. In addition the amount of 'pushing', an aggressive behaviour designed to move the rival away from the female, was performed in proportion to relative body size.

Thus, in principle, body size in *Triturus vulgaris* could affect interactions between males if smaller males are inhibited from interfering by the presence of larger rivals. A female preference for larger males may exist if size is an indicator of genetic quality or potential fecundity. In *Triturus vulgaris*, large males have larger testes and produce more spermatophores over a season (Verrell et al. 1986, Waights 1998). In causal terms, females may prefer larger males because their display is more effective.

Since crest height has been shown to affect female choice in *Triturus* (Chapter four), it would have been interesting to look at the relationship between crest height and mating success during interference. However, I wished to concentrate on the dynamics of competition at the *beginning* of the season (Chapter six) and the crest does not reach its maximum size for several weeks (Griffiths and Mylotte 1988). I therefore chose body size as the most probable influence on mating success during this period. Hedlund (1990) reported a significant correlation between male size and the probability of depositing at least one spermatophore at the beginning of the season, although no such correlation with pick-up was reported. Although Green (1991a) found that neither crest height nor snout-vent length had an effect on competition between males for access to females, the effect of these factors during interference was not investigated.

Aim: ***To investigate the effect of male size on sexual interference between males.***

Experimental hypotheses:

1. *There will be greater variation in male mating success among males of different sizes than among similar-sized males.*

2. *Larger males will obtain more matings than smaller males.*

5.6 Methods

Two groups of four tanks, each containing three males, were set up. In the first group (LMS), the tanks contained males of different sizes (large, medium and small) relative to each other, with a minimum of 3mm difference in snout-vent length (mean 4.6mm). In the second group (MMM), males were matched for size with less than 2mm difference in snout-vent length (mean 0.25mm).

Trials began when one female was introduced into each tank of three males. Courtship and interference were described in detail on to a hand-held tape-recorder, particular note being made of the identity of courting and interfering males and the outcome in terms of spermatophore deposition and pick-up. The female was removed after one encounter, defined as containing at least one deposition and terminated by one or more newts ascending to breathe or the female hiding in a refuge. After a break of at least one hour, a second trial was started with a different female. If a female failed to respond to male courtship after 20 minutes, she was replaced.

Two LMS and two MMM tanks were observed every day for eight days. Each group of males thus received a maximum of two receptive females on alternate days, resulting in a possible eight successful courtships per tank. In order to reduce the number of unmated females taken from the wild, each female was allowed to mate twice (on two separate occasions and in different tanks). The order in which virgin and once-mated females were introduced was varied between tanks to avoid order effects. The whole set of trials was

repeated three times although, due to a shortage of males, only two LMS and three MMM tanks were set up in the final week. The total number of tanks in each group was therefore 10 LMS and 11 MMM.

A settling period of two to three days was allowed before trials began. Females were housed under similar conditions to males but with a maximum of 20 to a tank with unmated and once-mated individuals kept separately. All tanks contained plenty of hiding places and weed was placed in the female tanks for egg-laying. Temperature remained between 10°C and 11°C throughout. The number of light/dark hours were adjusted weekly to coincide with London lighting-up times and varied from 10/14 hours to 15/9 hours. The light/dark cycle was manipulated so that 'dusk' occurred at 15:00 hours when the observation session began. The experiment took place between 6 March and 17 April, 1996.

5.7 Analysis and results

5.7.1 Male size

Five or more courtships were completed in nine tanks from each condition (LMS and MMM) and these were used in the following analysis. Four scores were obtained for each male:-

1. Mean number of depositions per encounter.
2. Mean number of inseminations per encounter.
3. Percentage of encounters in which females were courted to deposition.
4. Percentage encounters in which females were inseminated.

The mean (±SD) scores for each male 'size' were then calculated across the nine replications (Table 5.2). In the MMM tanks, males were arbitrarily named M1, M2 and M3.

Score	L	M	S	M1	M2	M3
Mean depositions/ encounter	1.2 ± 0.7	1.0 ± 0.8	1.0 ± 0.7	1.0 ± 0.8	1.4 ± 0.8	0.9 ± 0.5
Mean inseminations/ encounter	0.5 ± 0.4	0.5 ± 0.4	0.4 ± 0.3	0.5 ± 0.3	0.6 ± 0.6	0.5 ± 0.4
% encounters to deposition	53 ± 25	59 ± 35	53 ± 30	45 ± 30	59 ± 33	52 ± 30
% encounters with insemination	34 ± 21	29 ± 19	33 ± 21	38 ± 26	37 ± 30	37 ± 31

Table 5.2. Mean ± SD scores for each male 'size' across the nine tanks.

Examination of the data by eye was sufficient to see that there were no differences, either between males within a tank or between the two conditions. For statistical comparison, I used a Kruskal-Wallis test (data were not normally distributed) on the percentage of encounters with insemination for LMS and MMM tanks separately. There was no significant difference between males in either group (Statistics box 5.5A). It did not seem necessary to carry out statistical tests for the remaining scores since the results were clearly non-significant.

Some of the courtships included in the above analysis did not contain any interference. Since it is not known if the factors governing the occurrence of interference are the same as those determining the outcome of interference, the analysis was repeated using just those courtships which contained interference. All tanks with at least three encounters with interference were used (n = 10 in both groups). Once again there was no significant difference between males in either condition (Statistics box 5.5B).

Statistics box 5.5

Kruskal-Wallis to compare the percentage of encounters with insemination within tanks containing unequally and equally-sized males.

A. All courtships. LMS: KW = 0.14, df = 2, $p > 0.9$. MMM: KW = 0.001, df = 2, $p > 0.99$.

B. Courtships with interference only. LMS: KW = 1.63, df = 2, $p > 0.3$. MMM: KW = 1.67, df = 2, $p > 0.3$.

Although body size was not a contributing factor, there was variation in mating success among males and the extent of this variation differed between tanks. In some tanks, for instance, one male monopolised all the matings while in others males inseminated an equal number of females.

5.7.2 Further analysis: encounters with interference

Further analysis of the 41 LMS and 40 MMM courtships containing interference was carried out to elucidate some of the factors influencing mating success during interference. Such 'a posteriori' analysis must be treated with caution, but it was thought to be justified given the extensive amount of information available from these observations.

Prior to further analysis, data obtained from unmated and once-mated females were compared. There was no difference in the number of sequences per encounter ($t = 0.22$, $df = 16$, $p = 0.82$) or in the frequency distribution of courting males per encounter ($\chi^2 = 0.29$, $df = 2$, $p > 0.8$) as a function of the reproductive state of females. Data from unmated and mated females were therefore combined in subsequent analysis.

The nature and extent of interference differed between sequences within an encounter and sequences were thus the most appropriate components to use in analysis. Unfortunately, sequences did not provide independent data points and were therefore not suitable for statistical testing. In some instances, I have counteracted this problem by analysing data from all first, second, third and fourth sequences separately. Sequences were defined as the period of display leading to a single deposition and varied considerably in duration. For example, following deposition by a courting male, a rival male sometimes led the female away and deposited rapidly himself. This was counted as a separate sequence. Since body size had no effect on interference, data from LMS and MMM tanks were combined.

In addition to female mimicry (Verrell 1984a), interference was categorised as follows (Chapter four):-.

1. The female touched the male's tail but missed the spermatophore as a result of interference, either because she turned away or because she approached the interferer.
2. The female touched the male's tail and picked up despite interference.
3. The female turned away or turned to follow the interferer just before tail-touch. This invariably resulted in a failure to pick up.

Female mimicry occurred in only 6% of sequences (20% of courtships) with interference (Table 5.3) which contrasts strikingly with data from Verrell (1984a) who reported female mimicry in 82% of courtships with interference. No pick-up occurred during sequences with female mimicry or deposition without tail-touch.

	Tail-touch by interferer (female *mimicry*)	**Tail-touch by female**	**No tail-touch**
Number of sequences	19 (6%)	291 (90%)	14 (4%)

Table 5.3. Tail-touch during sequences with interference. Number (%) of sequences.

The majority of interference (90% in this experiment) involved tail-touch by the female, following which she approached the rival, turned away altogether or attempted to complete the encounter with the courter despite the interference. If the female approached the interferer, he either moved directly into creep or returned to retreat display. Encounters frequently contained sequences both with and without interference and with different types of tail-touch.

Pick-up was compared in sequences with and without interference (Table 5.4).

	Sequence one		**Sequence two**		**Sequence three**	
Interference	**With**	**Without**	**With**	**Without**	**With**	**Without**
Deposition	57	24	54	22	41	21
Pick-up	20	14	14	16	12	10
% Pick-up	35	58	26	73	29	45
Chi-squared (df = 1)	$\chi^2 = 3.73$, $p > 0.05$		$\chi^2 = 14.31$, $p < 0.001$		$\chi^2 = 2.02$, $p > 0.01$	

Table 5.4. Spermatophore transfer in sequences one – three, with and without interference.

Within each sequence, the results were obtained from a unique set of individuals and in this respect, were independent (although some tanks contributed more data than others). Spermatophore transfer was more successful in sequences *without* interference although this was only significant for sequence two and nearly so for sequence one (critical value for $\alpha 0.05 = 3.84$).

The way in which pick-up was achieved during interference was investigated further. Possible reasons for pick-up were categorised and quantified (Table 5.5). Less than half the pick-ups were obtained when the rival male was actually in the process of interfering. In all other cases, females picked up during a temporary absence of rivals.

Sequence	One	Two	Three	Four	Total	%
Rival male(s) in creep or breathing	1	2	3	1	**8**	**14.5**
Rival male(s) temporarily left behind	10	4	3	2	**19**	**34**
Rival males display together	3	1	3	1	**8**	**14.5**
Pick-up occurs despite interference	6	5	2	8	**21**	**37**

Table 5.5. Frequency of occurrence of different reasons for successful pick-up during sequences with interference.

Reasons why females may have *failed* to pick up spermatophores during sequences with interference were categorised in the same way (Table 5.6). 'Female off course' refers to situations in which the female moved directly towards the spermatophore but approached from an inappropriate angle (often because she had been jostled during interference). 'Failure to adhere' denotes situations in which the spermatophore did not stick to the cloaca, despite correct positioning of the female.

Sequence	One	Two	Three	Four	Total	%
Female approached rival	9	18	11	6	**44**	**43**
Female pushed by rival	4	5	7	4	**20**	**19**
Female off course	10	6	4	3	**23**	**22**
Failure to adhere	4	3	2	2	**11**	**11**
Female turned away	2	1	1	1	**5**	**5**

Table 5.6. Frequency of occurrence of reasons why females failed to pick up spermatophores during sequences with interference.

Approximately half of the spermatophores were missed as a result of direct interference, either because the female was attracted to, or was pushed by the rival. In about 22% of sequences, failure was due to incorrect positioning of the female, which may or may not have been the result of interference since it also occurred in the absence of rivals. In some instances, spermatophores were missed because they failed to stick to the female's cloaca. Occasionally the female turned away just before pick-up, possibly repelled by the interference. These observations cannot be used in statistical analysis because individuals contributed different amounts of data (see Machlis et al. 1985, for problems associated with the use of pooled data) but they do indicate an overall trend.

Table 5.7 compares the frequency of deposition and insemination by both courting and interfering males. As in chapter four, the courting and interfering males were defined as follows. If, *following deposition* by the courting male, the interferer succeeded in enticing the female into courtship with himself, he was named the courter for the next sequence. The previous courter could then interfere. If the interferer drew the female away *prior to deposition* by the original courter, there was no change of sequence and he remained the interferer. The male beginning a particular sequence was thus the courter (although this was not necessarily the male which displayed most frequently during the sequence).

	Depositions by courter	**Depositions by interferer**	**Inseminations by courter**	**Inseminations by interferer**
Sequence one	34	26	14 (41%)	6 (23%)
Sequence two	33	21	8 (24%)	6 (28%)
Sequence three	21	20	4 (19%)	8 (40%)

Table 5.7. Number of depositions and inseminations by courting and interfering males.

Deposition by the interferer was only marginally less common than by the courter and there was no difference in insemination success.

5.7.3 Further analysis: a comparison with data from the artificial pond

Courtships containing interference from the artificial pond study (1994 and 1995) were analysed for comparison with the laboratory data. The proportion of female mimicry and female tail-touch were broadly similar to those found in the laboratory (Table 5.8).

	Tail-touch by interferer (female mimicry)	Tail-touch by female	No tail-touch
1994	3 (7.5%)	34 (85%)	3 (7.5%)
1995	5 (16%)	22 (71%)	4 (13%)

Table 5.8. Tail-touch during sequences with interference. Number (%) of sequences.

Although an overall reduction in pick-up success during encounters with interference was observed in the artificial pond (Chapter four, Table 4.3) this was surprisingly not mirrored by a comparison between *sequences* with and without interference (Table 5.9). This may be due to the small sample sizes, in particular of sequences without interference.

		Sequence one		Sequence two		Sequence three	
Interference		**With**	**Without**	**With**	**Without**	**With**	**Without**
1994	**Deposition**	5	11	9	3	5	2
	Pick-up	0	6	1	0	0	0
	% Pick-up	0	54.5	11	0	0	0
1995	**Deposition**	10	5	4	2	2	2
	Pick-up	2	1	1	0	0	0
	% Pick-up	20	20	25	0	0	0

Table 5.9. Spermatophore transfer in sequences one to three, with and without interference during courtship in the artificial pond.

Again, in contrast to the results from the laboratory, deposition and insemination by the interfering male was very low in the artificial pond (Table 5.10). This has already been described in Chapter four (Table 4.8 and Table 4.2: note that Table 4.2 includes data from *all* sequences).

		Depositions by courter	Depositions by interferer	Inseminations by courter	Inseminations by interferer
1994	**Sequence one**	16	0	6	0
	Sequence two	8	4	1	0
	Sequence three	4	3	0	0
1995	**Sequence one**	13	2	3	0
	Sequence two	4	2	0	1
	Sequence three	4	0	0	0

Table 5.10. Number of depositions and inseminations by courting and interfering males during courtship in the artificial pond.

5.7.4 Female aversion to interference and other observations

Females have been reported to find interference 'aversive' (for example, Pavignano et al. 1993; Faria 1995; Sparreboom and Ota 1995) although the extent of this aversion depends on the stage of courtship reached prior to interference (Verrell 1984a). I categorised courtships according to the timing of interference and whether the female reacted negatively (refused to court) or positively (continued courtship) (Table 5.11).

Stage	Before creep		After creep		After 1st sequence	
Reaction	-ve	+ve	-ve	+ve	-ve	+ve
%	33	67	0	100	0	100

Table 5.11. The relationship between the timing of interference and female response. -ve: female refused to court. +ve: female continued to court.

If interference took place after the courter had reached creep in the first sequence, the females always continued to court. If interference began before creep, one third of the females swam away. Following this negative reaction, most females were eventually courted by a single male after the rival(s) had temporarily given up (73%) while a few continued to be courted by two or three males together and eventually began to respond positively to male display (27%).

A number of interesting observations were also made during the present experiment which could not be quantified but which are nevertheless worth reporting.

1. In situations in which two or three males displayed to the female simultaneously, she frequently responded by walking forward, thereby approaching the male that was currently directly in front of her. Males vied for that position, attempting to come between rival males and the female.

2. Display between males was common, even resulting in tail-touch and deposition. The introduction of a female appeared to increase the amount of display between males.

3. In situations of intense interference involving all three males, females were observed

attempting to follow any male which managed to move into creep. Spermatophore transfer, however, was constantly thwarted by the rivals. Under these circumstances it is unlikely that the female had the opportunity to discriminate between males.

4. There were other occasions in which it appeared that the female was able to exhibit a preference for a particular male. In seven encounters, a male which had not previously been involved in courtship appeared during a later sequence. The female made a positive approach towards him, ignoring those with which she had already mated. On one occasion, all three males deposited within thirty seconds and the female picked up from the last male while the others were still in brake. In another situation, two males deposited directly in front of the female who picked up from one of them. Lastly, one female approached a male which was depositing a spermatophore in response to tail-touch from a second male. The first male deposited and the female pushed between the two males to pick up the spermatophore.

5.7.5 Summary of results from experiment two

1. Male body size had no effect on male mating success during encounters with sexual interference. *Accept null hypotheses one and two that there is no difference in the variation in mating success between groups of equally and unequally-sized males and that small males obtain the same number of matings as large males.*

2. In the laboratory, tail-touch was instigated by the rival (female mimicry) in approximately 6% of sequences with interference, the female in 90% of sequences and the remainder contained no tail-touch. Data from the artificial pond revealed a broadly similar pattern. Pick-up only occurred following tail-touch by the female.

3. In the laboratory, pick-up was generally more successful during sequences without interference (although this was not always statistically significant). This trend was only partially supported by data from the artificial pond but sample sizes were small.

4. In half the cases of pick-up, rival males were temporarily absent. More than half the failures to pick up were a direct result of the interference, either because the female was attracted to or was pushed by the interferer.

5. In the laboratory, there was no difference between the number of inseminations by the courter and the interferer. During courtship in the artificial pond, however, the interfering male was much less likely to deposit for the female.

6. Female aversion to interference was observed in courtships in which the rival(s) approached before the courter had reached creep in the first sequence.

7. If several males displayed simultaneously, the female appeared to walk forward, approaching the male directly in front of her. Males vied for that position.

8. In a few situations it appeared that females had the opportunity to choose between males. At other times, such a choice seemed impossible.

5.8 Discussion

5.8.1 Male size

Male size had no effect on male mating success in *T. vulgaris*. Any preference by females for larger males or positive association between size and the effectiveness of display was not be expressed in a competitive situation. In contrast to *T. pygmaeus* (Reques and Halliday submitted) there appeared to be no inhibitory effect of large body size on the behaviour of smaller males. Perhaps such an inhibitory affect can only arise in species in which there is some aggressive behaviour between males. No direct aggression has been reported between male *T. vulgaris*.

Amphibians are a particularly interesting group in terms of the relationship between male size and mating success. Although a correlation between body size and mating success has frequently been reported in the literature (suggesting that sexual selection is important in the

evolution of body size), size is strongly influenced by life history processes which in turn are subject to considerable environmental variation (Partridge and Halliday 1984). In many species, sexual maturation is accompanied by a reduction in growth rate and the age at which this occurs depends on trade-offs between fecundity, growth and survival (Halliday and Tejedo 1995). Growth during the larval phase may, for example, be the most important contributor to variation in adult size (review in Halliday and Verrell 1988; smooth newts, Baker 1990).

Observed variation in a trait which is influenced by environmental factors, may be an expression of phenotypic plasticity rather than genetic adaptation (Berven 1982). Using reciprocal transplant experiments, Berven (1982; 1987) demonstrated that, in the wood frog, age and size at first reproduction were under both genetic and environmental control. Therefore, even if female preferences exist for large body size in amphibians, sexual selection is likely to play only a minor role in its evolution.

Although body size was not a contributory factor, variation in mating success occurred within some but not all of the tanks. More research is needed to understand the mechanisms behind this variation. For example, in juvenile *Anolis aneus*, persistence is more important than superior strength in territory settlement tactics (Stamps 1994). It is possible that the most persistent male smooth newt also gains most inseminations in the end.

5.8.2 Further analysis of interference

It was useful to examine the details of sequences with and without interference even though much of the data was not suitable for statistical analysis. However, the complexity and variety of behaviour patterns which resulted from interactions between groups of individuals often produced more questions than answers.

During sequences with interference, many depositions and, in particular, inseminations took place during the temporary absence of other males. Several questions arise with regard to this. *Why* did the other males get left behind? Were they lacking in motivation, more easily

distracted by rivals or was it merely a matter of chance? Did females wait for such an occasion to mate with their preferred male or did they take the first opportunity that arose?

The question of whether the opportunity for mate choice exists during interference is particularly important for future study. In some instances, females picked up the spermatophore despite interference and could theoretically have been expressing a preference for the courting male. On the other hand, there were situations in which the rival physically pushed the female away from the spermatophore, apparently preventing her from exercising any choice.

A number of experiments have demonstrated that a preference, observed under one set of circumstances may not be expressed in a different situation (Dyson et al. 1992; Halliday and Tejedo 1995). Verrell (1989b) introduced females singly and in pairs to male *Desmognathus ochrophaeus* and discovered that the male preference for larger females was only expressed when two different females were presented simultaneously. In view of the preference shown by females for higher-crested males when presented with single males, and since there is only a weak correlation between snout-vent length and crest height (Griffiths and Mylotte 1988), it would be interesting to repeat the present experiment using males of different crest heights, to establish if this preference can be maintained during interference. If a preferred male trait varies over the sampling period of the female (for example, crest height in male *T. vulgaris*) then it is important to consider the means by which that trait is assessed (Sullivan 1990). For example, females may choose males with the greatest *mean* value for the trait in question rather than the greatest value at a given moment.

More research is also needed into the process of interference from the female perspective. For example, does she distinguish between the 'courter' and 'interferer' or is she primarily attracted to the male directly in front of her? The female appears to be readily distracted by the display of a rival during spermatophore transfer and frequently (but not always) approaches the

new male. It would be useful to analyse the circumstances in which the reaction of the female is positive or negative to the interferer.

This experiment confirmed that females often react negatively to interference if it occurs during the early stages of courtship (Verrell 1984a). It also showed that interference can prevent a female from picking up a spermatophore even if she appears highly motivated. This supports the idea that the functional explanation of female aversion to interference is that it prevents her from mating with a preferred partner (Verrell 1984c). Unlike Verrell (1984a) however, I found that once the courter had reached the creep in the first sequence, females were willing to continue courtship for several successive sequences, despite interference. It is possible that the females in this study were particularly motivated since it was their first two courtships of the season.

How do the results from this experiment compare with those described in Chapter four from the artificial pond? Experimental and field data were consistent in demonstrating that, during interference, tail-touch was most frequently carried out by the female. This contrasts strikingly with data from Verrell (1984a) in which female mimicry (tail-touch by the rival male) was most commonly observed.

During the laboratory experiment, interfering males were no less successful than courting males in obtaining inseminations, in marked contrast to data from the artificial pond and from other field studies (Chapter four, Table 4.8). It is possible that interfering males were relatively successful in the laboratory because courtship took place in a simple, confined environment. In *T. vulgaris*, a male can adopt the role of both courter or interferer, depending on whether he encounters a single female or one already engaged in courtship. Such alternative strategies are characteristic of reproduction in urodeles (Halliday 1998). Courtship is likely to represent a preferred 'high-gain' strategy (Verrell 1984a), since it results in a greater rate of insemination. Interference can be described as a 'side-payment' strategy (Dunbar 1982), pursued when the

probability of finding and inseminating another female is less than the probability of achieving insemination during interference. This in turn will depend on the operational sex ratio (Chapter seven).

In Chapters two and three I suggested that the high insemination success, typical of laboratory experiments, was primarily due to the absence of interference between males. Although the overall pick-up rates in the current experiment were still relatively high, individual sequences with interference had a lower percentage pick-up than those without. Data from the artificial pond were less convincing but the results were based on a very small sample size, in particular sequences without interference.

The two experiments described in this chapter involved groups of eight or four freely interacting individuals and, in this respect, bridged the gap between experiments involving pairs of newts and the semi-natural population. The results contained characteristics of both laboratory and field data. For example, the high pick-up success in both experiments and, in particular, the number of sequences per encounter (median four and maximum nine) in experiment two did not accurately reflect courtship in the field.

On the other hand, the complexity and variety of behaviour patterns was much greater than that usually observed between two individuals. For example, courtship differed in terms of the type of interference (female mimicry, female tail-touch, no tail-touch), the number of sequences with and without interference, sequence length and the number of interfering males. Differences between individual males included motivation to court, degree of distraction by other males, persistence and display rate. Females differed in their receptivity to courtship and response to interference.

5.8.3 Conclusions

Figure 5.6 combines the main conclusions from Chapters four and five with those from Chapters two and three.

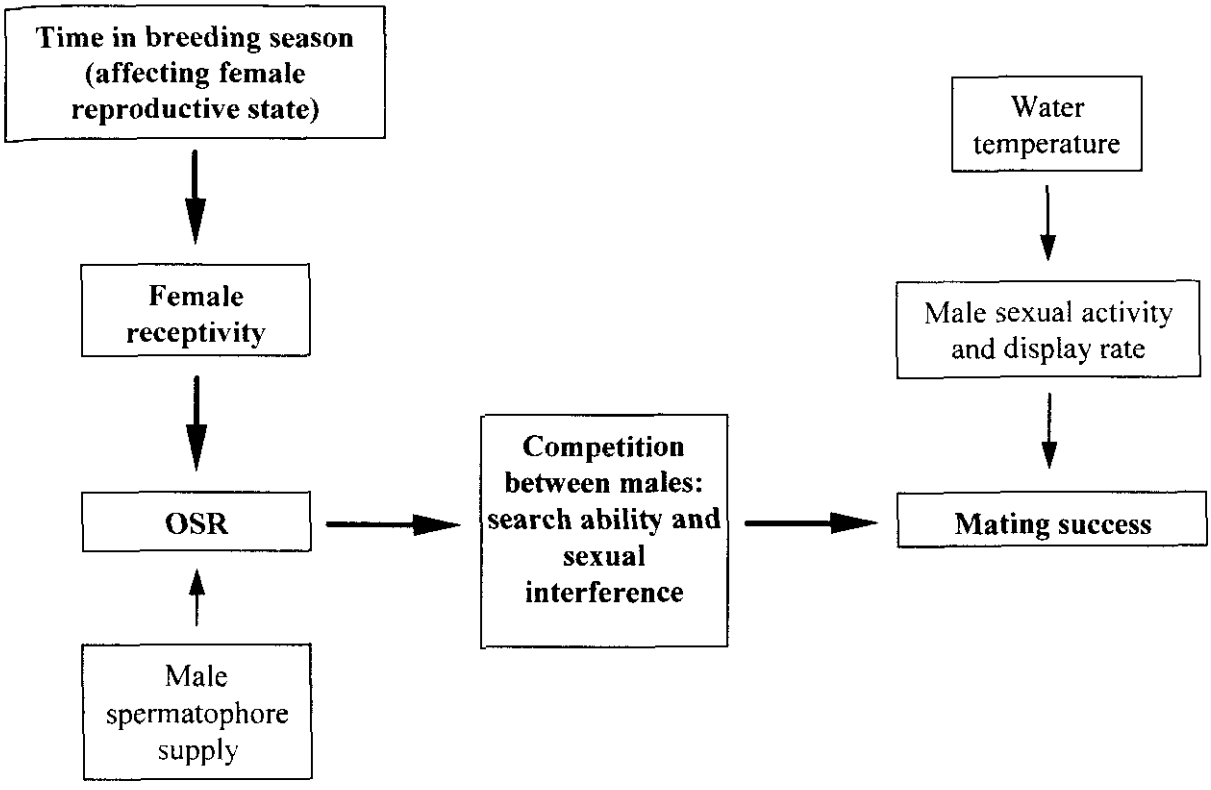


Figure 5.6. A summary of Chapters two to five: factors affecting the courtship and mating dynamics of the smooth newt. Bold indicates a stronger link.

In addition to the pattern of female receptivity (Chapter three), the operational sex ratio and competition between males (the ability to find receptive females and to compete through sexual interference) are key factors affecting mating success in the smooth newt. Chapter six investigates the extent of variation in immigration patterns and considers the effect on the operational sex ratio.

Chapter six

Immigration

6.1 Introduction

As discussed in Chapter four, the operational sex ratio (OSR) is one of the most important factors influencing the intensity of competition for mates and variance in mating success. The operational sex ratio is determined by the potential reproductive rate of males and females, the temporal and spatial distribution of the sexes and the adult sex ratio (Emlen and Oring 1977; Clutton-Brock and Vincent 1991).

It has already been demonstrated that the potential reproductive rate in smooth newts differs considerably between the two sexes. At the beginning of the season, females appear to be highly receptive and mate two or three times in quick succession. Following ovulation, receptivity is reduced and re-mating occurs only occasionally (Verrell and McCabe 1988; Hosie 1992; Chapters three and four). Males remain sexually active throughout the season but (in the laboratory) require 48 hours to replenish their spermatophore supply after being tested to sexual exhaustion (Verrell 1986b).

As a result of these differences in potential reproductive rate, it has been proposed that, prior to ovulation, female smooth newts are highly receptive to courtship and may outnumber sexually active males. There is the potential for female interference during this period and male interference is rare. Post ovulation, the OSR becomes strongly male-biased and competition between males for females is intense (Verrell and Halliday 1985a; Verrell and McCabe 1988; Waights 1996; Halliday 1998; Chapter four). Although I agree with the second part of this hypothesis, I believe that the first part of the season requires closer examination.

In the artificial pond (Chapter four), the number of depositions per encounter tended to be lower than that observed during laboratory experiments, reducing the expected depletion of male spermatophore supply at the beginning of the season. In addition, the *pattern of immigration* to the breeding site will have a significant impact on the operational sex ratio.

Immigration patterns are determined by the adult sex ratio, the duration of the breeding period (breeding synchrony) and sex differences in the timing of arrival.

In smooth newts, as in many species of urodeles, it is likely that some individuals do not attempt to breed each year (Halliday and Tejedo 1995; Halliday 1998). It is therefore necessary to define the *breeding sex ratio* (distinct from the adult sex ratio) as the ratio of males to females arriving at the breeding site. The best estimates of breeding sex ratio are obtained using capture data from drift fences, since sampling within the pond often leads to errors due to different habitat preferences of males and females. However, since a number of individuals may overwinter in the pond (Bell 1977) the drift fence data may also not be wholly accurate. There appears to be considerable spatial and temporal variation in the breeding sex ratio within the genus *Triturus* (Verrell and Halliday 1985a).

The terms explosive and prolonged breeder were introduced by Wells (1977) in his influential paper on anuran social behaviour and are now widely used in descriptions of amphibian mating systems. Explosive breeders usually complete courtship and egg-laying within days or weeks. few males have the opportunity to mate more than once and there tends to be little variance in male mating success. The breeding season of prolonged breeders lasts from a few months to all year round. At any given moment, the availability of receptive females tends to be low, providing the opportunity for a few males to monopolise many females (Wells 1977). Wells emphasised that these definitions should not be interpreted too rigidly since they are used to describe points along a continuum of mating systems.

The effect of breeding synchrony may be mediated by the breeding sex ratio. In many explosive breeders, for example, variance in male mating success occurs as a result of strongly male-biased breeding sex ratios, when some males fail to mate at all (Berven 1981; Arak 1983). With a breeding season of several months, the smooth newt could be categorised as a prolonged breeder. However courtship does not occur regularly throughout the season, but is

most frequent for a short period following the arrival of unmated females (Verrell and McCabe 1988; Chapter three). The duration of the arrival period will therefore have a crucial effect on male competition for females during the first part of the season.

In order to maximise mating success, males must time their reproductive effort to coincide with maximum female availability (Thornhill and Alcock 1983) and in a wide range of species, males are ready to mate shortly before the arrival of receptive females. This may involve early arrival at the breeding site, the prior establishment of territories or early emergence from hibernation (Michener 1983; Reynolds et al. 1986; Arak 1988). Protandry (the emergence of males before females) is particularly common among insects (for example, solitary bees, wasps, butterflies and mosquitoes, Wiklund and Solbreck 1982; Thornhill and Alcock 1983). In sex role-reversed species such as red-necked and Wilson's phalaropes, females are the first to arrive at the breeding grounds (Reynolds et al. 1986; Clutton-Brock and Parker 1992).

Darwin (1874) discussed sex differences in the timing of reproductive activity in migratory birds but also referred to fish, frogs and insects: 'In many cases special circumstances tend to make the struggle between the males particularly severe. Thus the males of our migratory birds generally arrive at their places of breeding before the females, so that many males are ready to contend for each female.' He noted that while selection could produce early arrival among males, the same was not true of females which were constrained by the appropriate conditions for raising young.

The spatial distribution of the sexes may also be important in determining the operational sex ratio. Small-scale local variation in the OSR can arise where there is incomplete mixing of the population. For example, Healy (1974) demonstrated that female *Notophthalmus viridescens* preferred shallow water, while males were more frequently found in the deeper areas. This was not tested during the present study, however, and is not discussed further.

This chapter uses data from the drift fence at Braystone farm, from two other ponds and from the literature to examine the variation in the breeding sex ratio and arrival patterns of *Triturus vulgaris*. Chapter seven uses a model to investigate the potential effect of such variation on the OSR, mating success and sexual selection.

Aim: ***to investigate variation in the breeding sex ratio and immigration patterns of smooth newts to their breeding pond over three years at Braystone Farm and to compare this with data from other ponds.***

Specific questions:

1. *What is the variation in breeding sex ratio?*

2. *What is the duration of the arrival period?*

3. *Are there differences between the sexes in the timing of arrival?*

6.2 Methods

The drift fence, erected at Braystone Farm, Aspley Guise was described in Chapter two. In 1994, the traps were checked daily between 26 February and 14 June when the fence was dismantled. By this date it was clear that the newts had begun to leave the pond in significant numbers. In 1995, the fence was checked daily between 1 February and 12 May when it was evident that no more animals were entering the pond. Since I was primarily interested in arrival data, it was unnecessary to wait until the animals were caught leaving the pond and risk harming other species which could fall into the traps. In 1996, the same approach was used and the fence was erected between 20 January and 26 April.

Richard Griffiths (Llysdinam pond in South Wales) and Pim Arntzen (Corn Close pond in Leicestershire) very kindly gave me access to their raw data so that I could examine and compare the details of immigration at two other ponds. All their data were collected using drift fences.

6.3 Analysis and results

6.3.1 Breeding sex ratios and arrival patterns

Cumulative arrival of males and females at the drift fence, Braystone Farm, is shown in Figure 6.1.

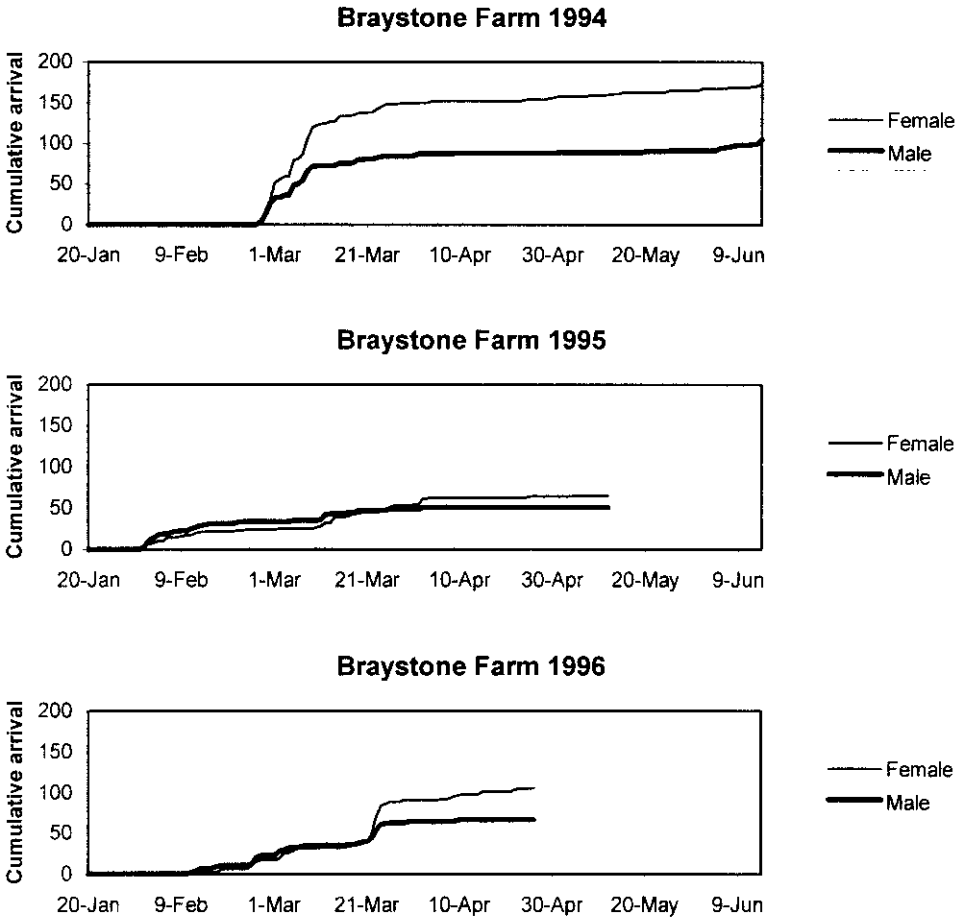


Figure 6.1. Cumulative arrival of smooth newts at the drift fence, Braystone Farm, 1994 – 1996.

The difference in density of the breeding population between years was marked, total numbers in 1995 being reduced to only a third of those in 1994. In all years, the overall breeding sex ratio was female-biased and although immigration *began* at the same time in both sexes, there was variation in the timing of arrival. In 1994, there was a higher proportion of females from the start of the season and the median date of immigration (by which 50% of individuals have arrived, Griffiths et al. 1986) was the same in both sexes. In both 1995 and 1996, males were more numerous at the beginning of the immigration period and the median

date of arrival of males preceded that of females by four weeks in 1995 and two weeks in 1996.

Differences in the duration of the immigration period between years are illustrated more clearly by percentage cumulative arrival (Figure 6.2).

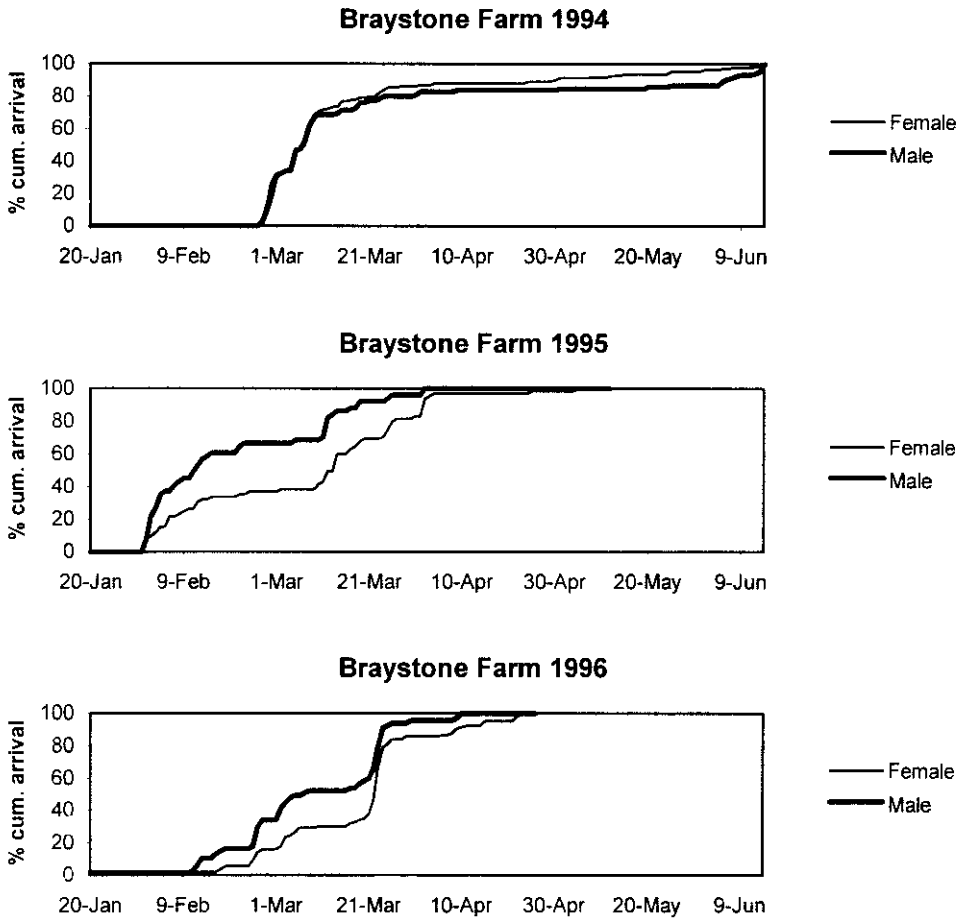


Figure 6.2. Percentage cumulative arrival of smooth newts at the drift fence, Braystone Farm, 1994 – 1996.

Immigration occurred relatively rapidly in 1994 with almost 80% of the population present after the first three weeks. In this year, bad weather prevented the fence from being erected until 26 February and therefore these data must be treated with some caution. However, few individuals are likely to have entered the pond while the ground was snow covered. In contrast to 1994, arrival was much more gradual in 1996 and was intermediate in 1995.

The arrival patterns of smooth newts at Llysdinam and Corn Close ponds are compared in Figure 6.3.

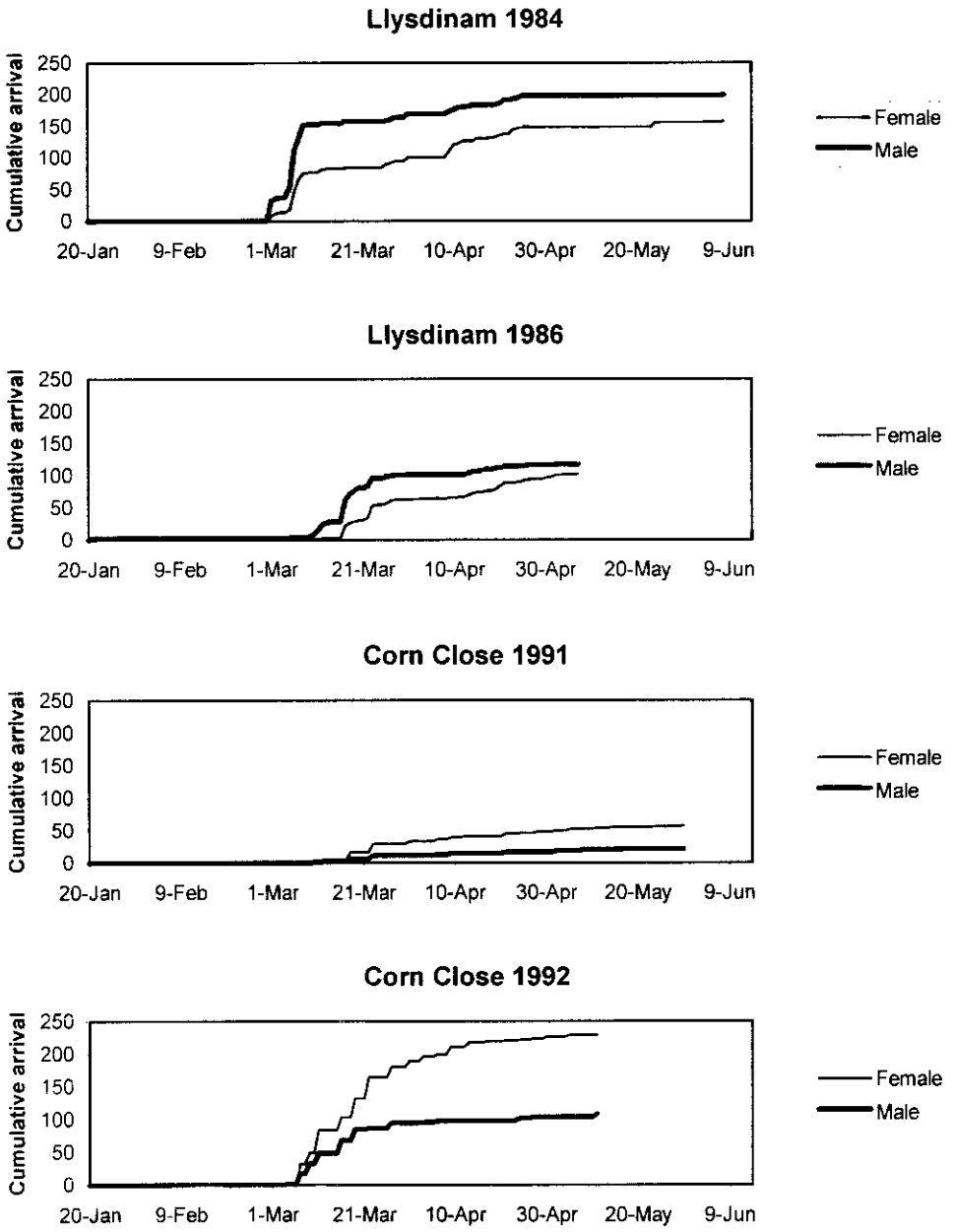


Figure 6.3. Cumulative arrival at Llysdinam and Corn Close ponds (kind permission of Richard Griffiths and Pim Arntzen).

Once again, there were considerable differences in breeding population densities between years. The breeding sex ratio was male-biased at Llysdinam and female-biased at Corn Close. At Llysdinam, immigration began simultaneously in 1984 (with males in greater numbers) while in 1986, males started to arrive about one week before females. The median date of

arrival of males preceded that of females by six days in both years. At Corn Close, the pattern was similar to that seen in Braystone Farm in 1994, males and females arriving together with a higher proportion of females from the start of the season. The median dates of arrival were similar in both sexes.

The duration of immigration is illustrated by percentage cumulative arrival (Figure 6.4).

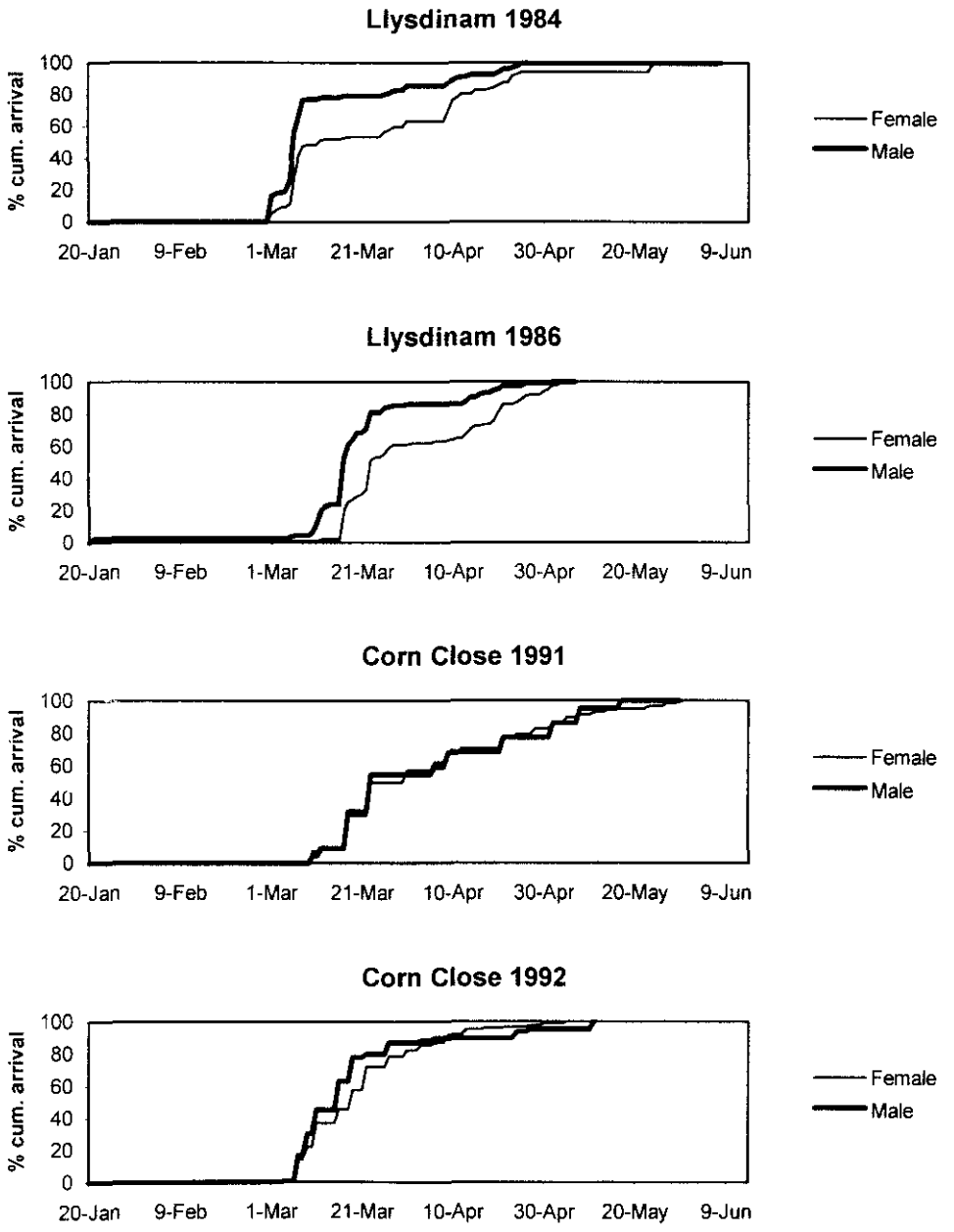


Figure 6.4. Percentage cumulative arrival at Llysdinam and Corn Close ponds.

Immigration occurred rapidly at Llysdinam in 1984 with 40 – 60% of the population arriving within the first week but was more gradual in 1986. At Corn Close arrival was more gradual in 1991 than in 1992.

The sex ratio can be visualised graphically by expressing the proportion of males out of one hundred: (100/ total number of males and females) × number of males (Kvarnemo and Ahnesjö 1996). Fifty therefore represents unity, numbers below 50 denote a female bias and numbers above 50 a male bias. 100 and 0 represent males only and females only respectively. In order to illustrate the variation in sex ratio during arrival, the cumulative sex ratio was calculated over the first 21 days of immigration (regardless of the actual date) (Figure 6.5). Two years from Braystone Farm (1994 and 1996) and one from Llysdinam were chosen (1984) to represent different immigration patterns. The overall sex ratios were female-biased at Braystone Farm but in 1996, males were more numerous at the beginning of the season. Llysdinam represents a male-biased sex ratio.

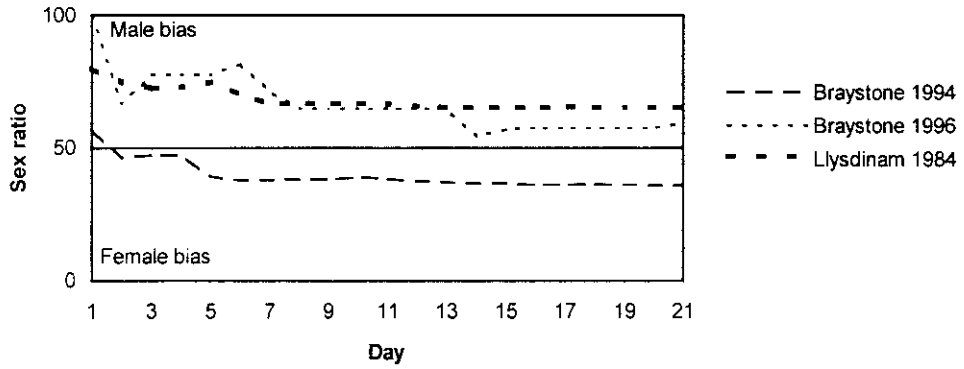


Figure 6.5. Cumulative sex ratio over the first 21 days of arrival. The horizontal black line at a sex ratio of 50 represents unity. For calculation of sex ratio see text.

The cumulative sex ratio is clearly dynamic, changing considerably even within the first three weeks of the season. Sex ratios which have been averaged over large portions of the breeding season are therefore of limited use in estimating the effect of the OSR on mating dynamics.

In order to understand the small scale impact of different adult sex ratios and arrival patterns, it is helpful to look at the actual numbers of males and females arriving each day over the first three weeks. The same three examples have been used (Figure 6.6). The data indicate that there are distinct 'female arrival' and 'male arrival' nights, suggesting that each sex may be responding to different combinations of climatic variables.

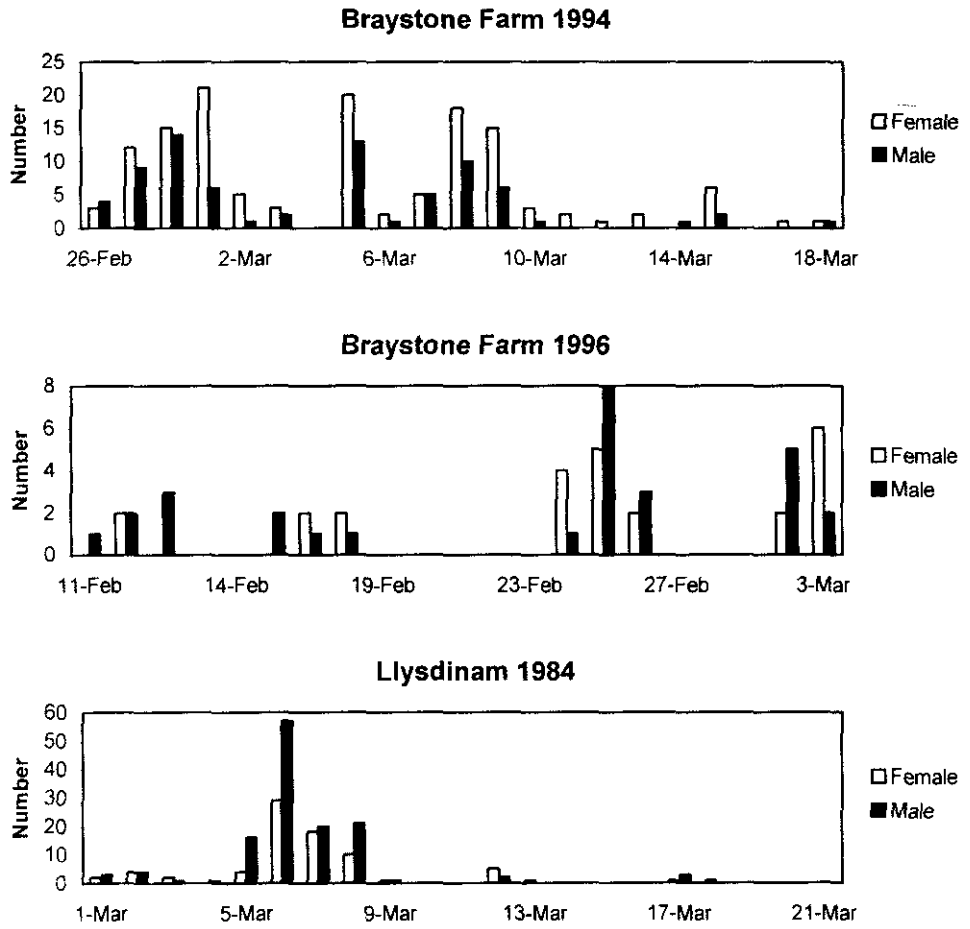


Figure 6.6. Numbers of males and females arriving over the first three weeks.

The OSR will be affected, not only by the numbers of males and females arriving on a particular night, but by arrival patterns over the preceding few nights (Figure 6.6). For example, competition between males was probably negligible during the first few days at Braystone Farm 1994, since females were arriving in greater numbers. Indeed, this situation may have been conducive to female interference. On the other hand, males arriving on 25 February 1996 with an apparent excess of females, would face competition from earlier male

arrivals. Male-male competition was clearly much higher at Llysdinam where males arriving on 17 March would almost certainly have found no remaining receptive females.

Both males and females require some time to adjust to the aquatic environment and become sexually active. The impact of the arrival of new individuals to the pond on the mating dynamics will therefore be delayed by a few days. In interpreting Figure 6.6, I am assuming that there is no significant difference between males and females in the time taken to enter the pool of sexually active individuals. Griffiths (1984) proposed that one reason for the early arrival of males was to allow time for development of secondary sexual characteristics before the arrival of females. However, courtship has frequently been observed, both in the laboratory and in the wild, before male crests are fully developed (Hosie 1992; Halliday 1998, this thesis).

6.3.2 Summary of results from Chapter six

1. The breeding sex ratio of *Triturus vulgaris* varied from female-biased to male-biased.
2. There was considerable variation in the duration of the arrival period.
3. In some populations, males arrived before females while in others, both sexes arrived simultaneously.

6.4 Discussion

The main purpose of this chapter is to illustrate the variation in breeding sex ratio and arrival patterns of the smooth newt. Table 6.1 compares the data presented above with similar records for *Triturus vulgaris* from the literature (all of which were obtained using drift fences). The *first* record of a migrating individual is often inaccurate. If newts are caught immediately the fence is erected, some individuals are likely to have been missed during the preceding period (see Kauffmann 1994; Arntzen 1991, 1992; Harrison et al. 1983 in Table 6.1). On the other hand, fences erected very early may catch one or two individuals before migration can be said to have begun (Verrell and Halliday 1985a). Where possible, I have used the first date

after which there was no break in immigration exceeding two days to represent the start of migration.

Male immigration		Female immigration		Sex ratio (m:f)	Location and Authors
Start date	**Median date**	**Start date**	**Median date**		
26/2/94	7/3/94	26/2/94	7/3/94	1:1.65	Braystone Farm, Kauffmann
1/2/95	12/2/95	1/2/95	13/3/95	1:1.3	
11/2/96	7/3/96	12/2/96	23/3/96	1:1.6	
1/3/84	6/3/84	1/3/84	12/3/84	1:0.8	Llysdinam, Griffiths
5/3/86	17/3/86	12/3/86	23/3/86	1:0.87	
10/3/91	23/3/91	10/3/91	23/3/91	1:3.5	Corn Close, Arntzen
7/3/92	16/3/92	7/3/92	19/3/92	1:2.1	
5/3/83*	19/3/83	5/3/83*	19/3/83	1:2.06	Bucks, Verrell and Halliday 1985a
5/2/81*	6/3/81	Not specified ***	8/3/81	1:2.6	Llysdinam, Harrison et al. 1983
20/2/76*	4/3/76	21/2/76*	10/3/76	1:1**	Bonn, Blab and Blab 1981
16/2/77*	5/3/77	8/2/77*	10/3/77	1:1**	

Table 6.1. Summary of arrival patterns and sex ratios in *T. vulgaris*, using the raw data from this chapter plus accounts from the literature. * These dates were given as the first capture date but it is not known if this was followed by a break in immigration exceeding two days. Sex ratio refers to the overall ratio of immigrating newts. ** Period over which sex ratio was calculated is unknown. *** Harrison et al. (1983) state that although males arrived slightly before females, the overall difference in timing between the sexes was relatively small.

6.4.1 Breeding sex ratio

The data presented in Table 6.1 suggest that a female-biased sex ratio is most common for *T. vulgaris* but this is by no means a consistent finding. For example, in their paper which summarised five years of data from Llysdinam pond, Griffiths et al. (1986) found the average sex ratio of *T. vulgaris* to be 1:1.3 (m:f) but there were two years with a male-biased sex ratio. Bell (1977) in his pioneering work on the smooth newt found a female-biased sex ratio in *most* of his 35 study ponds.

There is also considerable variation in the breeding sex ratio of other *Triturus* species. Both male-biased and female-biased sex ratios have been observed in *T. alpestris* (male-

biased, Blab and Blab 1981; female-biased, Faber 1994) and female-biased sex ratios have been reported for *T. helveticus* (Harrison et al. 1983; Griffiths et al. 1986). Sex ratios in *T. cristatus* vary from male-biased to female-biased (male-biased, Blab and Blab 1981; male-biased, Hedlund 1990; unity, Hagström 1979; unity, Verrell and Halliday 1985b; female-biased, Bell 1979 in Verrell and Halliday 1985b). The existence of female-biased sex ratios in *Triturus* is in marked contrast to most other urodele genera. For example, a male-biased sex ratio has been reported for *Ambystoma jeffersonianum* (Douglas 1979), *A. maculatum* (Husting 1965), *A. texanum* (McWilliams and Bachmann 1988), *A. tigrinum* (Sever and Dineen 1978; Semlitsch 1985), *Notophthalmus viridescens* (Healy 1974; Gill 1978), *Taricha granulosa* (Propper 1991) and *Taricha torosa* (Halliday unpubl. data), for additional papers see Verrell and Halliday (1985a). Several authors also remarked upon the considerable spatial and temporal variation in breeding sex ratios in these species (Hurlbert 1969; Gill 1978; Faber 1996).

It is not clear why the breeding sex ratio of *Triturus vulgaris* should frequently be female-biased. Factors which may influence the breeding sex ratio include a difference in the relationship between environmental cues and the timing of male and female migration (Douglas 1979; Henzi et al. 1995), a higher mortality of one sex during juvenile or adult stages, a difference between the sexes in the length of time spent at the breeding site (Halliday and Tejedo 1995), delayed reproduction by one sex (Partridge and Endler 1987; Clutton-Brock and Parker 1992), or a difference between the sexes in the frequency of reproduction following maturation (Griffiths 1984; Halliday and Tejedo 1995). Within the amphibia, for example, females appear to miss breeding opportunities more frequently than males, although the mechanisms which determine whether a female breeds in a given year are not well understood (Halliday and Tejedo 1995). It should be noted that while the first factor is directly related to climate, all other factors are life-history variables which tend also to be strongly influenced by

environmental conditions. All of these explanations could apply to *T. vulgaris* and more research is clearly needed.

6.4.2 The duration of the arrival period

The data presented in this chapter and Table 6.1 also demonstrated considerable variation in the duration of the period of arrival of *T. vulgaris*, with a range in the number of days from the start to median dates of immigration of between 9 and 41(average 20) for females and between 6 and 29 (average 15) for males. Within the immigration period, the number of individuals arriving each night was also highly variable.

Many authors agree that climatic conditions during the spring and preceding winter are likely to exert a major influence on migration to the breeding site, although there is less agreement on the precise mechanism by which this influence is exerted on amphibians. Immigration has been correlated with minimum air temperature (Verrell and Halliday 1985a, *T. vulgaris*; Verrell and Halliday 1985b, *T. cristatus*), accumulated temperature (Beattie 1985, Rana temporaria), maximum temperature (Beebee 1995, *T. vulgaris*), rainfall (Hurlbert 1969, *Notophthalmus viridescens*), air temperature and rainfall (Husting 1965, *Ambystoms maculatum*; Douglas 1979, *A. jeffersonianum*; Hardy and Raymond 1980, *A. talpoideum*; Harrison et al. 1983, *T. vulgaris*), and water temperature and rainfall (Brodman 1995, *A. maculatum*). A number of authors have noted the temporal and spatial variation in patterns of immigration (Husting 1965; Hurlbert 1969).

In a detailed analysis of the effect of climatic factors on the migration of *Ambystoma talpoideum* (a winter breeding species from southern USA), Semlitsch (1985) concluded that factors affecting the onset of migration were different from those determining the length and magnitude of migration into the pond. For example, the onset of migration was associated with the coldest months of the year but was not related to a threshold temperature or cumulated rainfall. The length of the period of immigration, on the other hand, was associated with

rainfall; intense rain over a short period producing a short period of immigration and sporadic rain over a longer period producing a more extended immigration pattern. Finally, daily variation in movement was related to both temperature and rainfall, the greatest number of individuals moving on nights when rain coincided with a high minimum air temperature. Semlitsch remarked that the high degree of local and annual variation in the timing of migration would have a profound influence on the ability of males to find and compete for females. Faber (1994) also recognised that the period over which immigration took place was dependent on climatic factors. For example, an early start to migration in a high altitude population of *T. alpestris* followed by a return to freezing conditions produced a long, drawn-out immigration period, whereas a delayed start to migration encouraged all individuals to arrive within the space of three weeks.

Climate may thus affect not only the timing of male and female immigration but also the number of individuals moving on any one night and the population density. The striking differences in annual population density observed at Braystone Farm has been described in other populations of *Triturus vulgaris* and *T. helveticus* (Griffiths et al. 1986). Variability in population density may be a characteristic of many amphibian populations. During a 16 year study of the amphibian community at Rainbow Bay (Savannah river, South Carolina), fluctuations in breeding population sizes of three orders of magnitude were recorded (Pechmann et al. 1991; Semlitsch et al. 1996). Breeding population size was correlated with rainfall (fewer adults arriving to breed in years with low rainfall) and with juvenile recruitment. Recruitment was highly episodic, recruitment failure again being closely related to the occurrence of drought (Pechmann et al. 1991; Semlitsch et al. 1996).

6.4.3 Differences between the sexes in the timing of arrival

The difference between the sexes in the timing of arrival was again variable in *Triturus vulgaris* (Table 6.1). At some study sites, males arrived before females while in others, both

sexes moved together. Most other studies of *Triturus* have reported males arriving, on average, before females; *T. helvetivus* (Blab and Blab 1981; Harrison et al. 1983), *T. cristatus* (Blab and Blab 1981; Verrell and Halliday 1985b), *T. alpestris* (Faber 1996). However, this is marginal in some populations (Verrell and Halliday 1985b; Faber 1996).

Early male arrival may be the result of direct selection for males to maximise mating success or it may be the indirect result of selection for other life history traits (Wiklund and Solbreck 1982). Thornhill and Alcock (1983) used a model to demonstrate that direct selection for protandry in insects is likely to occur in species with once-mating females in which males have equal competitive ability. The proportion of males emerging before females rises as male reproductive lifespan increases relative to the period of female receptivity. Males which are sexually active throughout the mating period should be ready to mate just before females become receptive (Thornhill and Alcock 1983). If males require additional time to prepare for reproduction, the difference in timing between the sexes is correspondingly increased. The model may equally well be applied to other taxa. In the newt *Taricha torosa*, for example, males arrive before females with sufficient time to swell in size through water absorption, a factor which affects their competitive ability (Halliday 1998).

With the exception of the refractory period needed for short-term spermatophore replenishment, male smooth newts are sexually active throughout the period of female receptivity and, according to the model of Thornhill and Alcock (1983), are expected to arrive before females. Several factors may help to explain the variability in early male arrival in *Triturus vulgaris*. First, female multiple mating should moderate the strength of selection for early male arrival, particularly in species exhibiting last male paternity (Thornhill and Alcock 1983; Halliday 1998). However, in *T. vulgaris*, a large proportion of courtship takes place early in the season and it would still appear advantageous for males to be ready to mate with newly-arriving females.

Secondly, the advantages of early arrival may be mediated by female-biased sex ratios since the intensity of competition between males is reduced. Sex ratios among the genus *Triturus* are highly variable (see above), contrasting with the strongly male-biased breeding sex ratios of other urodele genera, such as *Ambystoma* and *Taricha*, in which early male arrival is the overriding pattern (Douglas 1979; Hardy and Raymond 1980; Semlitsch 1985; Halliday 1998). Once again, this explanation is not fully satisfactory since the *operational sex ratio* may soon become male-biased even within female-biased breeding sex ratios (Chapter seven).

Thirdly, there may be severe costs associated with early arrival at the breeding site. A shortage of food, for example, affects both crest development (Green 1991a) and the production of spermatophores (Halliday et al. unpubl. data). There is also a greater probability that the pond will be frozen or will re-freeze, with the associated risk of oxygen shortage and 'winterkill' (Verrell and Halliday 1985c). Fourthly, early male arrival would not be beneficial if courtships obtained later in the season were more successful, for example, as a result of sperm competition or improved offspring survival.

Finally, it is important to remember that a difference in the timing of arrival between the sexes is not a character upon which selection can act. Selection for early arrival in *females* (for example due to the protracted period of egg-laying and the risk of pond-drying) would reduce the relative differences in arrival times.

The proximate mechanisms behind early male emergence are not fully understood. Either there must be separate processes controlling male and female immigration or males and females must differ in their response to the same cues. In both cases, the mechanism which determines the movement of males must anticipate that of females (Michener 1983; Thornhill and Alcock 1983) which is particularly difficult if the arrival of females is unpredictable (Reynolds et al. 1986).

Within the amphibia, however, there is some evidence that the arrival of males and females at the breeding site may be determined by different combinations of environmental cues. For example, male *Ambystoma jeffersonianum* tend to arrive at their breeding pond earlier and in greater numbers than females (Douglas 1979). Females wait for a prolonged period of favourable conditions before migrating 'en masse' to the pond and the numbers of migrating females is stable between years. Males, on the other hand, start to migrate as soon as minimal conditions are met and risk encountering poor weather conditions during the migratory period. As a result, males exhibit greater annual fluctuations in arrival patterns. Douglas (1979) recognised that there was a direct relationship between the operational sex ratio and changes in the temporal distribution of males and females, resulting from small climatic differences between years.

Henzi et al. (1995) used a GLM model to assess the amount of variance in chorus attendance of male and female painted reed frogs. They used a null model, the numbers of the opposite sex and a wide range of environmental factors as independent variables. Most variation in male and female attendance was predicted by different sets of environmental cues, possibly reflecting different selection pressures on the two sexes.

The data presented in this chapter also suggest that there were distinct 'female arrival' and 'male arrival' nights. Future research should concentrate on the relationship between climatic variables and arrival patterns by focusing on males and females separately. The GLM model would be a useful approach but would need to take the sex ratio of the population into account and the fact that individuals may travel different distances to reach the pond.

6.4.4 Conclusions

This chapter has demonstrated marked variation in breeding sex ratios, arrival patterns and population densities of the smooth newt. As a result, the operational sex ratio is likely to vary both within and between seasons, as different numbers of males and females arrive at the

breeding site. In general terms male-biased populations, and female-biased populations in which males arrive before females, are expected to have a *male-biased operational sex ratio* from the beginning of the season. However, the degree of male bias will be mediated by the duration of the female arrival period. If females arrive 'en masse' the intensity of competition between males should be reduced as more receptive females are shared between sexually active males. The reverse will apply if female arrival is gradual or sporadic. In this way, smooth newts may exhibit the characteristics of either explosive or prolonged breeders (or both), depending on the length of the immigration period. *A female-biased operational sex ratio* may, in principle, arise in a female-biased population in which the sexes arrive simultaneously (similar start *and* median dates of arrival).

More research is needed to clarify the mechanisms which control immigration in urodeles. Nevertheless, to a large extent, variation in the breeding sex ratio, arrival patterns and breeding population densities will be determined by life-history events (under both environmental and genetic control) and directly by environmental factors. The next chapter uses an individual-based model to quantify the effect of different arrival patterns on the operational sex ratio and variation in mating success and discusses the extent to which the observed variance in mating success can be attributed to stochastic or deterministic elements.

Chapter seven

The model

7.1 Introduction

Clutton-Brock and Parker (1992) demonstrated the central role of potential reproductive rate in determining the operational sex ratio (OSR) but acknowledged that it did not account for fluctuations in the OSR in space and time. Two important influences in this respect are the breeding sex ratio and the spatial and temporal distribution of the sexes (Emlen and Oring 1977). In the light of the wide variation in breeding sex ratio (ratio of adult males to females entering the aquatic phase) and arrival patterns of the smooth newt, described in the preceding chapter, I decided to investigate the relationship between these factors, potential reproductive rate, the OSR and mating success in more detail.

As discussed in Chapter four, variance in mating success indicates that the opportunity for sexual selection exists, provided there is a link between mating success and one or more phenotypic traits. However, the strength and direction of sexual selection may be compromised if there is a strong environmental component to variance in mating success (Clutton-Brock 1988; Halliday and Tejedo 1995). Within the mating dynamics of the smooth newt, such an environmental component may operate via its influence over immigration patterns and the operational sex ratio (Chapter six).

It would have been ideal to measure the OSR and variance in mating success in the field or laboratory. The difficulties associated with field observation of urodele sexual behaviour, however, have already been emphasised (Verrell 1989a; Chapter three). In the case of the smooth newt, courtship occurs at dusk in murky, weed-filled ponds. Even in the laboratory, an experiment to compare variance in mating success over a range of sex ratios would have required an unrealistic sample size. In particular, unmated females (which are responsive to courtship) are difficult to obtain in large numbers. In addition, the OSR (which in smooth

newts is determined by the ratio of males with available spermatophores to receptive females) cannot be manipulated by the experimenter and is difficult to measure in the field (Sullivan et al. 1995). The use of a model solves not only the problems of observability and sample size, but also allows direct manipulation of the potential reproductive rate of males and females, thus permitting precise calculation of the OSR at any instant in time. For reasons outlined below and in Appendix one, I decided to use an individual-based, object-oriented computer simulation.

7.1.1 Individual-based modelling

The individual has been at the core of the modelling of behaviour for many years. In particular, situations in which the actions of one individual depend on the strategies adopted by competing individuals (frequency-dependent decisions) have been studied using game theory and ESS models (Maynard Smith 1982; Krebs and Davies 1993). These models were developed from the principles of natural selection in which each animal is assumed to maximise its own fitness (optimisation theory) (Mangel and Clark 1988). Traditionally, modelling in ecology has attempted to find general equations to describe large-scale systems (for example. Rose 1987) often involving the assumption that all individuals react in the same way and inhabit a uniform environment Over the last twenty years, the link between behaviour and ecology has strengthened (Lomnicki 1992) and with it the understanding that larger scale processes are essentially driven by the decisions of individuals. From the 1970s onwards, ecological models began increasingly to follow a new approach which emphasised the role of the individual within population processes (for example, Thompson et al. 1974; Shugart et al. 1980; Saarenma et al. 1988; Shugart 1990; DeAngelis and Godbout 1991; Huston 1992). The key points behind this approach are:-

1. Individuals are unique. They do not respond uniformly to a particular environment (Huston et al. 1988).

2. Interactions are local. The environment experienced by one individual is not necessarily identical to that of another (Huston et al. 1988).

3. 'The properties of any system are derived from the properties of its parts and the relations between them'. (Lomnicki 1992).

4. Many small decisions produce complex behaviour. Small differences in individual characteristics and in the local environment can have a major effect on the outcome for an individual, and on the larger scale processes to which that individual contributes (chaos theory) (May 1976; Huston et al. 1988).

The main principles of individual-based models are that the characteristics of an individual change as a function of its current state, and its interactions with other individuals and with the local environment (Maley and Caswell 1993). This type of modelling has a number of advantages: it is conceptually straightforward, being composed of simple rules which direct the interactions between individuals; the rules affect properties which can be tested directly, thus involving fewer assumptions than conventional modelling (Huston et al. 1988; Murdoch et al. 1992); it allows the inclusion of rare events or rare individuals into the modelling system (Gross et al. 1992) and it elucidates the importance of individual variation, rather than attempting to eliminate it (Crowder et al. 1992; Lomnicki 1992).

Disadvantages of such models include the difficulty of interpreting the complexity which arises from numerous interactions and the specificity of the models to the organisms under study (although many of the rules are widely applicable, Murdoch et al. 1992). Individual-based models also require data sets obtained from detailed observations of individuals throughout their lifetime, although collaboration between behaviourists and ecologists have facilitated the transfer of this type of data (Lomnicki 1992). As a result, individual-based models are most suited to the study of small populations, living in heterogeneous environments, and are particularly useful for establishing the effects of small changes in local

dynamics (sensitivity analyses) and for pinpointing suitable areas for future research (Gross et al. 1992).

The importance of this approach began to be stressed during the 1990s. Following a conference on the subject, DeAngelis and Gross (1992) edited a book which outlines various methods and applications for individual-based modelling and contains several new examples (for example, Crowder et al. 1992; Real et al. 1992). Excellent overviews are given in Huston et al. (1988), Judson (1994) and Uchmanski and Grimm (1996). There has also been an increasing emphasis on the role of individual behaviour in behavioural and population ecology in the wider literature. Some authors have emphasised the importance of studying variation among individuals (for example, Lott 1991). Sutherland (1996) devoted a whole book to the link between individual behaviour and population ecology, and individual behaviour is featured in the latest edition of, 'Behavioural Ecology' (Krebs and Davies 1997).

It is necessary to clarify the use of some terms in modelling. Within ecology, individual-based modelling has been applied to many different approaches including analytical models which use equations to represent aggregates of individuals (Gross et al. 1992). These are often known as i-state distribution models (i-state referring to the attributes of individuals). Most authors, however, agree that the term individual-based is synonymous with i-state configuration models in which each individual is considered as a discrete entity (Maley and Caswell 1993). Within behavioural research, models (although often centred around individuals) tend to be given more specific terms, such as game theory, optimisation, or search-time models.

Simulation is another widely used term with a rather vague meaning. Maynard Smith (1974) contrasted simulations with models. Simulations were used in the analysis of highly specific, detailed, often complex scenarios whereas models attempted to find simple, abstract and general mathematical statements with wide applicability. More recently, the term

simulation has been used for all individual-based models which represent individuals as discrete entities (Gross et al. 1992) or as *computer* models which track individuals (Huston et al. 1988). These are not clear distinctions, since simulations can be conducted without computers and there are simulations of i-state distribution models (Maley and Caswell 1993).

There is no doubt that improvement in computer technology has been immensely influential in the recent focus on individual-based modelling and most models do take advantage of computers to simulate interactions between individuals. Computer simulations can be approached in two contrasting ways. Procedural programming tends to operate in a linear fashion, following predetermined routes though the code in which the data structures and their control routines are treated separately. Object-oriented programming, on the other hand. identifies discrete objects which contain both properties and the rules governing those properties and allows interactions to take place with no fixed order. In their summary paper, (Gross et al. 1992) it was reluctantly concluded that the programming language used in simulation models was important, but there were no clear directives concerning which software or hardware to use. Since then, the object-oriented approach to programming has more frequently been discussed (Maley and Caswell 1993; Judson 1994) but, apart from a few exceptions (Saarenma et al. 1988; Folse et al. 1989; Sequeira et al. 1991), seems rarely to have been put into practice.

The newt model described in this chapter falls somewhere between classic ecological and behavioural models. The model asks questions about behaviour (mating success) at the level of the individual but is also concerned with the impact of the local environment. In this respect the individual-based modelling approach is ideal. Since object-oriented programming was used in the newt model, I shall enlarge on the basic features below. For full details, see Appendix one.

7.1.2 Object-oriented programming

One of the most exciting aspects of object-oriented programming is that, in many ways, the structure of the program is analogous to a living system. The program is based around a system of objects which can communicate with one another (Palmer 1992). Each object is defined by a set of properties and behaves according to a set of rules. The objects can convey information about themselves in response to questions and can thus interact with one another and with their environment on a local scale. Objects develop or behave differently according to their unique experiences during simulation (Folse et al. 1989).

Objects exist at many levels of a hierarchy. As in taxonomy, an object at a lower level will inherit the properties and the rules governing its ancestors as well as adding new elements itself. The object-oriented approach is particularly appropriate for individual-based models (Judson, 1994: Maley and Caswell, 1993; Sequira et al., 1991) in which the individuals themselves are the objects, interacting in a realistic way.

Aim: ***to investigate the influence of the breeding sex ratio, male reproductive rate and arrival patterns on the operational sex ratio, competition between males and variance in mating success.***

Specific questions:

1. *How does the breeding sex ratio affect the operational sex ratio, competition between males and variance in mating success*? A male-biased breeding sex ratio is predicted to result in a male-biased OSR, competition between males and variance in male mating success. Females should exhibit little variance in mating success. A female-biased sex ratio, on the other hand, may produce a female-biased OSR in which there is variance in female mating success but competition between males is reduced.

2. *What effect does a reduction in male spermatophore supply have on the operational sex ratio, competition between males and variance in mating success*? A reduction in the spermatophore supply of males is expected to lower the ratio of sexually active males to

receptive females, reducing the amount of competition between males and the variance in male mating success but increasing the variance in female mating success.

3. *What is the effect of a gradual versus simultaneous arrival at the breeding site on the operational sex ratio, competition between males and variance in mating success*? In a population which arrives gradually, the males arriving first will have the opportunity to replenish their spermatophore supplies and court with additional females. This should result in a more strongly male-biased OSR, greater competition between males and increased variance in male mating success. Variance in female mating success should be reduced.

7.2 Methods

7.2.1 The basic hierarchy

A brief overview of the program structure is presented here while full details are given in Appendix one. The model is based on a hierarchy of objects (Figure 7.1). Objects lower in the hierarchy inherit the properties of the preceding objects while adding some specific properties of their own. Objects interact with each other according to a set of rules, the implementation of which depends on the values of the properties.

The model defines an environment object within which all other objects function. The model boundary determines a fixed three-dimensional space (the pond) and the term *entity* applies to every object that exists within the pond (Figure 7.1). Entity is divided into passive entities and active entities. Active entities have additional properties which give them the ability to move. Active entities are further divided into male and female entities with properties and rules specific to their sex. Male and female entities represent individual newts, while passive entities represent objects which contribute towards the complexity of the environment such as plants or rocks.

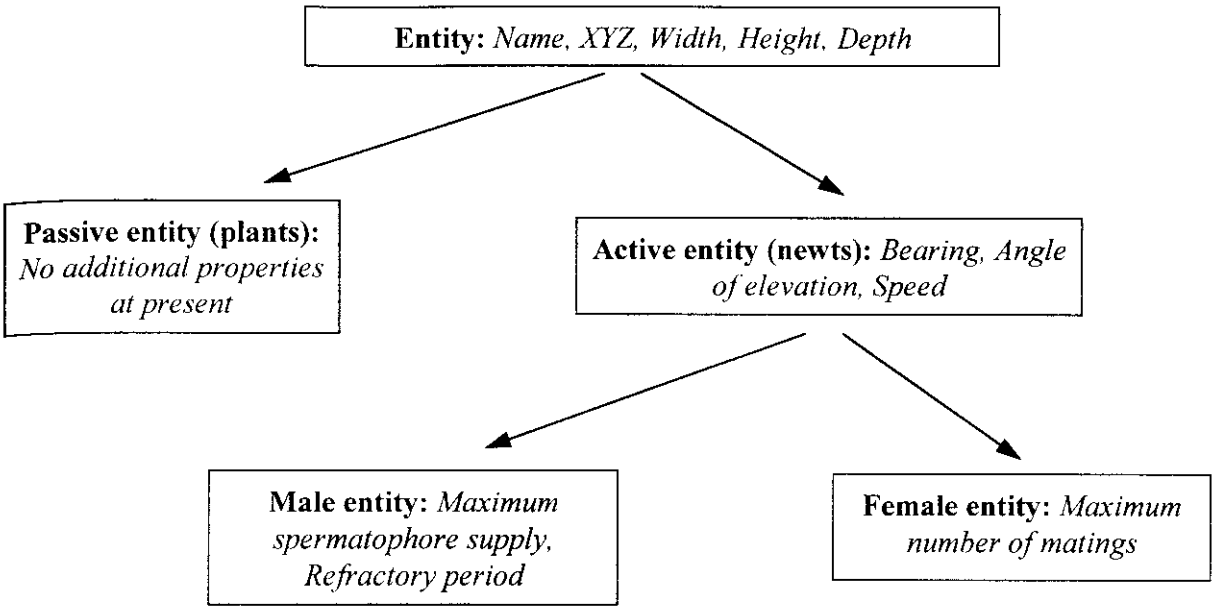


Figure 7.1. The basic hierarchy of objects and their main properties.

7.2.2 How does the model work?

The model clock (which exists within the environment object) pulses every second. At every pulse, each individual (male or female entity) calculates its position in relation to every other active and passive entity. The individual makes its next decision based on this information. Each active entity has an initial bearing, angle of elevation and speed which allows it to move within the model space. Changes in these variables are determined by laws which avoid collisions with other entities and restrain the newts within the model boundaries. The direction of movement can also be affected by behaviour such as attraction towards another newt.

The remainder of the model was designed specifically to simulate sexual activity in smooth newts in order to study the relationship between immigration patterns, reproductive rate, the operational sex ratio and variance in mating success. A male is attracted to any female which comes within his *sensory range*. He increases his speed and changes bearing in order to

follow the female for a set period of time. After this period, three questions are asked which determine how the male proceeds:-

1. Does the male have any spermatophores?
2. Is the female receptive?
3. Is the female already being courted by another male?

Each male is given an initial set of spermatophores. Once these spermatophores have been deposited he enters a refractory period (representing the period of spermatophore replenishment) during which he is unable to court. Following the refractory period, he obtains a new set of spermatophores. The answer to the first question thus depends on the state of the male, determined by the current value of the following properties:-

1. *Spermatophore supply*: the initial number of spermatophores assigned to each male.
2. *Male refractory period*: the duration of the refractory period.
3. *Spermatophore supply decrease*: after each refractory period, males receive a new spermatophore supply which may contain fewer spermatophores.

Females engage in a given number of courtships before becoming unreceptive. For the purposes of the model, one courtship is defined as any encounter resulting in at least one deposition. In the wild, female receptivity is more likely to be influenced by the number of courtships containing *pick-ups*. However, as previously emphasised (Chapters two, four and five), the mechanisms controlling pick-up are still poorly understood and it was not possible to incorporate pick-up into the model in a realistic way. The answer to the second question thus depends on the state of the female, which is determined by the current value of the property: *maximum number of courtships*.

If the answer to the third question is no and *provided the female is receptive and the male has a supply of spermatophores*, the newts stop moving and courtship begins. Courtship is divided into four periods. The first period represents static and retreat display during the first sequence. The second period is the first spermatophore transfer phase, ending with the first deposition. Period three represents a short second sequence leading to the second spermatophore deposition and period four represents display and deposition during the third sequence. At the end of courtship, the courter loses his attraction to the female and the newts resume their original speed and direction.

If the answer to the third question is yes, the male will proceed, not with courtship but with *interference*. The effect this has on deposition depends on the stage of courtship reached (see Appendix one for details). The encounter is always terminated at the end of the period in which interference occurs and both males lose their attraction to the female. The maximum number of spermatophores which can be deposited per encounter is three (see Appendix one for justification) but fewer depositions will take place if courtship is interrupted by interference or if the male has no remaining spermatophores. For the sake of simplicity, only two males are allowed to court a female at the same time and a male cannot be attracted to the female he has courted most recently.

The maximum or initial values for each property are assigned to individuals in an input file which is read into the model at the beginning of each simulation. For details of all values used in the following simulations see Appendices one and two. The values of properties most relevant to courtship behaviour are described below (section 7.2.3).

7.2.3 The simulations

Sensitivity analyses involve assessing the impact created by altering a single variable within a more complex system. The model was used to carry out a series of sensitivity analyses

in order to investigate the effect of changing the following variables on the mating dynamics of the smooth newt:-

1. Breeding sex ratio (the ratio of males to females entering the aquatic phase)
2. Male reproductive rate (in the form of spermatophore supply)
3. Arrival patterns

The results were expressed by four measurements, obtained at various time intervals during the simulations:-

1. *Females per male*: the number of females for whom each male deposited at least one spermatophore (regardless of whether the male was in the role of courter or interferer).
2. *Courtships per female*: the number of encounters obtained by each female in which at least one spermatophore was deposited.
3. *Courting males per female*: the number of males (both courters and interferers) which attempted to court each female, regardless of whether deposition occurred or not.
4. *Operational sex ratio*: the ratio of males with remaining spermatophores to females with remaining courtship opportunities.

Since pick-up was not included in the model, it was necessary to use courtships containing depositions as the measure of mating success. For the present, I am therefore making the unrealistical assumption (Chapter four) that within the population, pick-up occurs as a constant proportion of deposition. As soon as there is more information on the occurrence of pick-up, this can be inserted into the model. With this proviso, *females per male* represents male mating success, *courtships per female* represents female mating success and *courting males per female* indicates the extent of male competition for females.

Breeding sex ratio

In order to compare simple changes in sex ratio, a series of simulations were carried out with a population of 20 individuals at the following ratios (m = males: f = females):- 8m:12f, 9m:11f, 10m:10f, 11m:9f, 12m:8f. After analysing the first set of results, I added another female-biased ratio; 7m:13f. The population was introduced simultaneously and the simulations ran for three hours to represent an average period of courtship per night.

Male reproductive rate

Within the model, male reproductive rate can be altered by varying the maximum number of spermatophores or the length of the refractory period. Since the value given to the maximum number of spermatophores per male in the above simulations (eight) was probably a generous estimate (Appendix two), I chose to investigate the effect of altering this variable to six. A population of 20 was introduced simultaneously and the simulations were run for three hours. Three sex ratios were used; female-biased (8m:12f), unity (10m:10f) and male-biased (12m:8f).

The duration of the arrival period

In order to compare a relatively long and short arrival period, another set of simulations was carried out with a population of 40 individuals. Three sex ratios were used: female-biased (16m:24f), unity (20m:20f) and male-biased (24m:16f). The populations were added gradually over four nights (asynchronous) or simultaneously (synchronous) (Table 7.1).

		Asynchronous arrival				**Synchronous arrival**
		Day one	**Day two**	**Day three**	**Day four**	**Day one**
F-biased	**M**	4	4	4	4	16
	F	6	6	6	6	24
Unity	**M**	5	5	5	5	20
	F	5	5	5	5	20
M-biased	**M**	6	6	6	6	24
	F	4	4	4	4	16

Table 7.1. The number of males and females introduced within three sex ratios during synchronous and asynchronous arrival at the pond. M: male, F: female.

Properties and their values

The following properties were most crucial to courtship behaviour. All input values given below were held constant except spermatophore supply, which was altered in the simulation investigating reproductive rate.

1. Spermatophore supply = eight. This was considered representative of males at the beginning of the season. See Appendix two for the experiment from which this value was obtained.

2. Male refractory period = 10800 seconds (three hours). Since the simulations were run for three hours per night, this represented one night (Appendix two). The refractory period was not used in the single night simulations.

3. Spermatophore supply decrease = two. Each new spermatophore supply, following the refractory period contained two fewer spermatophores (Appendix two).

4. Maximum number of courtships = three. Data from the artificial pond (Chapter four) and Hosie (1992) suggests that females court two or three times at the beginning of the season, before the start of the egg-laying period.

Replicate number

Throughout this chapter, I shall refer to a *simulation* as a complete scenario with a unique set of values, population size and sex ratio and a *replicate* as one run of the simulation. In the simulations described in this chapter, each replicate contained individuals which had been placed randomly within the model space and were therefore considered to be independent. All measures (such as mean, median, standardised variance) of the above scores were therefore calculated from the population as a whole (all replicates pooled).

The number of replicates obtained for each simulation is equivalent to the sample size of an experiment. I carried out some tests to determine an appropriate replicate level in which a low variance among replicates was balanced by a reasonable time taken to run the simulation.

A simulation with a population of 20 individuals and the sex ratio at unity was carried out with 10, 20 and 30 replicates. This was repeated three times in order to obtain a coefficient of variation for each number of replicates. The score used was the number of *females per male*.

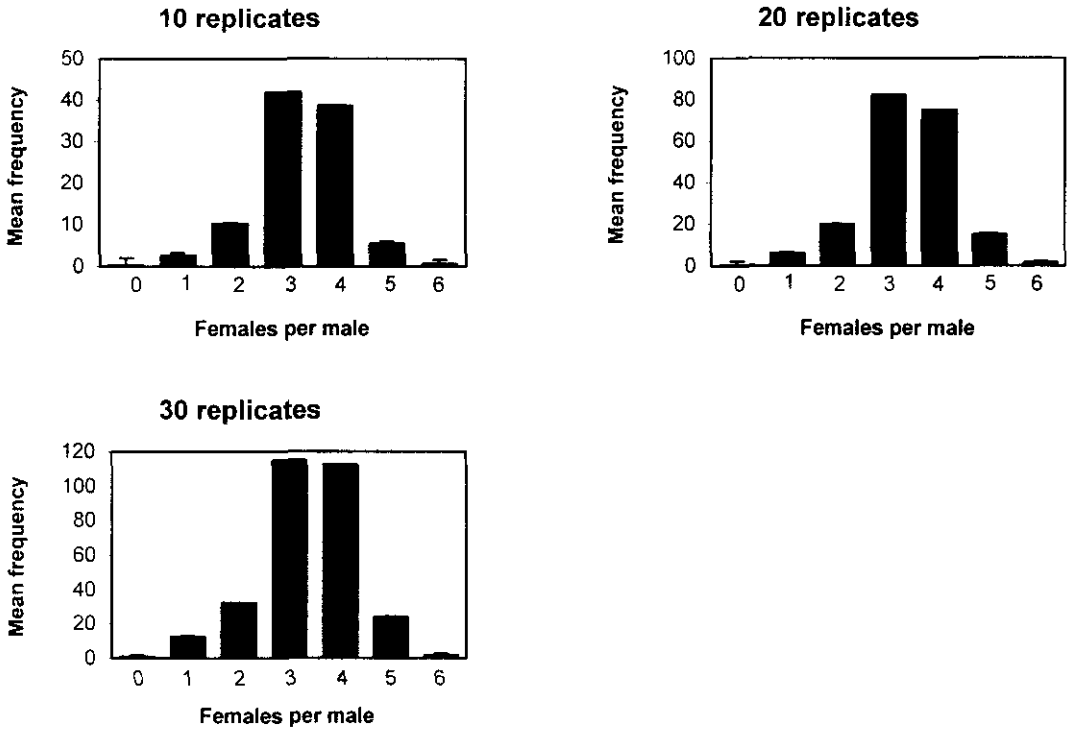


Figure 7.2. Mean frequency of *females per male* from three trials with either 10, 20 or 30 replicates. Error bars represent the coefficient of variation. Population number was 20 with the sex ratio at unity.

The overall distribution was extremely stable whether 10, 20 or 30 replicates were used (Figure 7.2). However, the coefficient of variation among trials was lower with 30 replicates in each than with 10, in particular at the more extreme values (0, 1, 5 and 6 females). Thirty was considered to be the maximum number of replicates which could be handled by the available computers and was used throughout the following simulations.

7.4 Analysis and results

7.4.1 Breeding sex ratio

How did the *operational sex ratio* change over time at different breeding sex ratios? The OSR was defined as the ratio of males with remaining spermatophores to females with

remaining courtship opportunities. As described in Chapter six, sex ratios can usefully be quantified as the proportion of males out of 100 and, using this method, the breeding sex ratios were 60 (12m:8f), 55 (11m:9f), 50 (10m:10f), 45 (9m:11f), 40 (8m:12f) and 35 (7m:13f). One hundred represents a situation in which there are no more receptive females available whereas zero represents the opposite situation in which all males have depleted their spermatophore supply.

The OSR was calculated every 30 minutes. Although the simulations ran for three hours, one or other sex had always used up all mating opportunities within two hours and Figure 7.3 includes 0 – 120 minutes only.

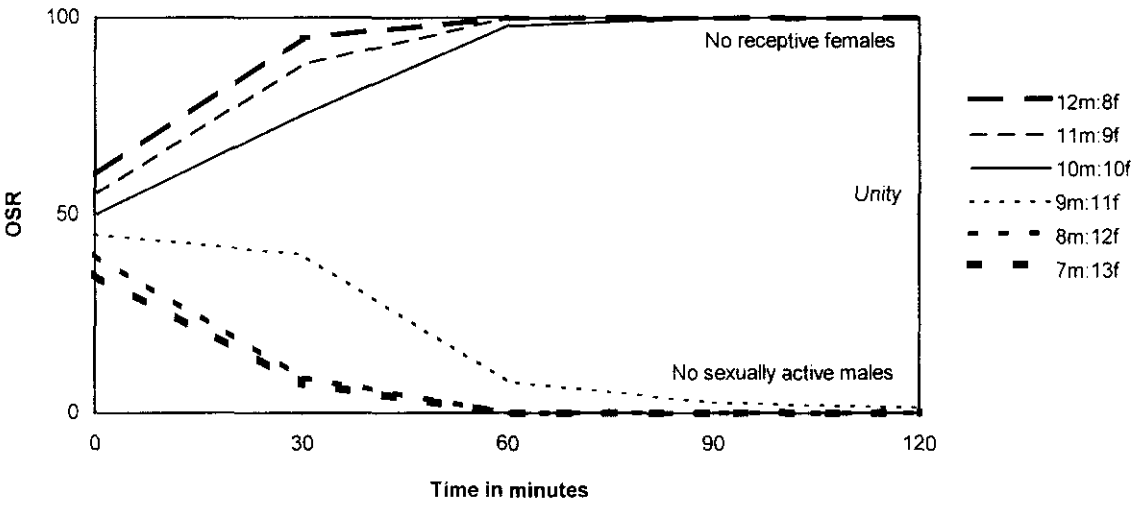


Figure 7.3. Changes in the operational sex ratio over time during simulations with different breeding sex ratios. Results pooled from 30 replicates (600 individuals) at each sex ratio.

The four most extreme breeding sex ratios (12m:8f, 11m:9f, 8m:12f and 7m:13f) led to rapidly increasing male- and female-biased OSRs. The results for the two central sex ratios, however, are particularly relevant. An initial sex ratio of unity developed an increasingly male-biased OSR but less rapidly than the male-biased breeding sex ratios. A slightly female-biased breeding sex ratio, on the other hand, developed an increasingly female-biased OSR but failed to reach an OSR of zero (male spermatophore supply entirely depleted) since in one of the 30

replicates of this simulation (9m:11f) female mating opportunities were used up before male spermatophore supply.

What was the effect of breeding sex ratio on variance in mating success and how did this relate to the amount of competition between males? At the end of each three-hour simulation, I calculated the mean, standard deviation and variance for the number of *females per male* and *courtships per female* (Figure 7.4, A to D). As in Chapter four, I used the standardised variance ($\sigma^2/\bar{x}^2$) which represents the opportunity for selection (Wade and Arnold 1980; Partridge 1988). Since there was no upper limit to the number of males which could attempt to court each female, the distribution of *courting males per female* was highly skewed (with a long right tail). I calculated the median and interquartile range for *courting males per female* (Figure 7.4E). A single value for each measurement was obtained directly from the total population of 600 individuals (all 30 replicates pooled) at each sex ratio.

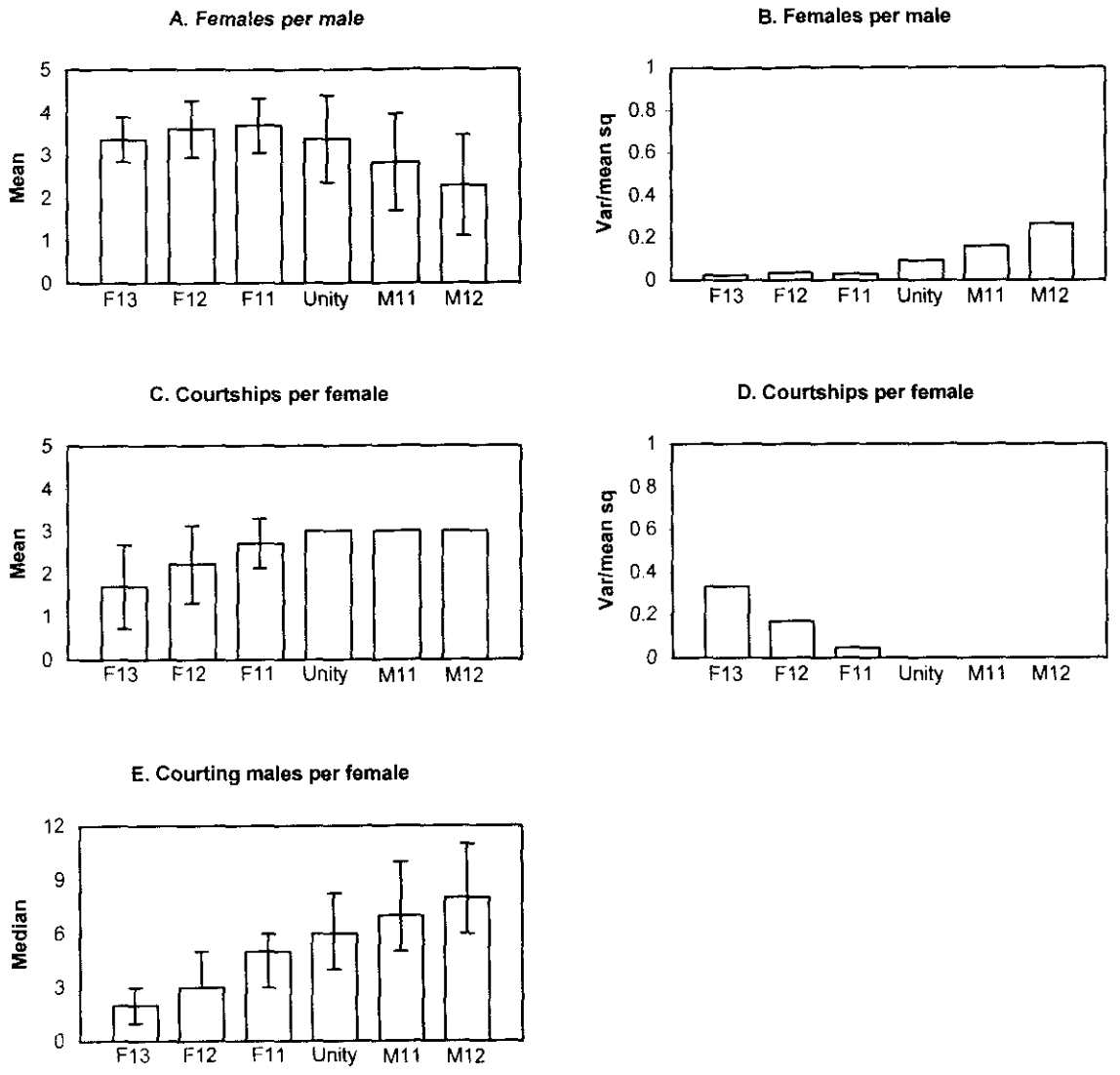


Figure 7.4. Mean (±SD) and standardised variance in *females per male* (A and B) and *courtships per female* (C and D) and median (± interquartile range) of *courting males per female* (E) at different sex ratios. F13 = 7m:13f, F12 = 8m:12f, F11 = 9m:11f, Unity = 10m:10f, M11 = 11m:9f, M12 = 12m:8f. Results pooled from 30 replicates (600 individuals) at each sex ratio.

At the female-biased sex ratios, the mean number of *females per male* was relatively high (approximately 3.5) and variance low. The number of *courting males per female*, representing the amount of competition between males, decreased as the proportion of females in the population increased. The surprising reduction in mean *females per male* at the most female-biased ratio (F13) can be explained by the low incidence of interference at this sex ratio. Most males would have deposited the maximum of three spermatophores per encounter, distributing

all eight of their spermatophores between just three females. The mean number of *courtships per female* decreased and variance increased with an increasingly female-biased sex ratio since many females did not receive the maximum of three courtships before each male's spermatophore supply was depleted.

As the number of males increased through unity to a male-biased ratio, mean *females per male* decreased while the variance increased. This was accompanied by a rise in the number of *courting males per female*. Thus, as competition for females increased, the difference between the most and the least successful males became more marked. There was no variance in *courtships per female* at unity and male-biased sex ratios since all females achieved the maximum of three courtships.

Overall, there was a significant positive correlation between an increasingly male-biased sex ratio and the variance in *females per male* and a significant negative correlation between an increasingly male-biased sex ratio and the variance in *courtships per female*. The difference in the number of *courting males per female* between sex ratios was also significant (Statistics box 7.1).

The percentage frequency distribution of *females per male* (Figure 7.5) and *courtships per female* (Figure 7.6) give a more detailed picture of the changes taking place at different sex ratios.

Statistics box 7.1

Spearman's rank order correlation between sex ratio and the variance in *females per male* (one-tailed). $r_s = +0.94$, $n = 6$, $\mathbf{p < 0.02}$.

Spearman's rank order correlation between sex ratio and the variance in *courtships per female* (one-tailed). $r_s = -0.83$, $n = 6$, $\mathbf{p < 0.05}$.

ANOVA on log transformed data (log10(x+1)) to compare *courting males per female* at different sex ratios. $F = 394.7$, $df = 5$, $\mathbf{p < 0.001}$.

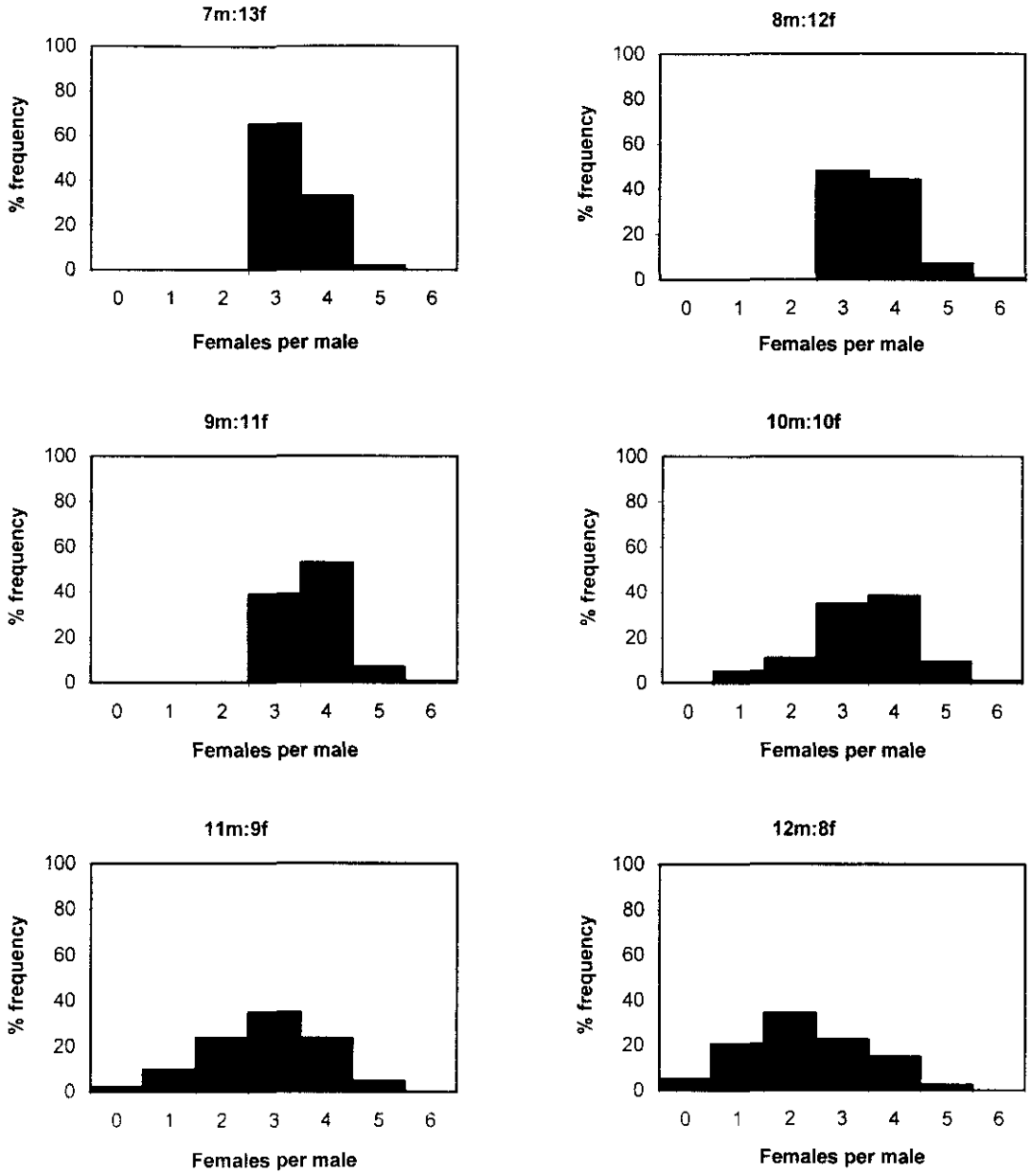


Figure 7.5. Percentage frequency distribution of *females per male* at six different sex ratios. Results pooled from 30 replicates (600 individuals) at each sex ratio.

An increasingly female-biased sex ratio was accompanied by a shift to the right in the number of *females per male* and by a narrower distribution. As the number of females increased but the incidence of interference decreased, most males distributed their spermatophore supply between three or four females. There was a highly significant

correlation between sex ratio and the frequency distribution of *females per male* (Kendall's tau, Statistics box 7.2).

With the exception of the most strongly female-biased sex ratios, the distribution of *courtships per female* was clustered to the right as the majority of females obtained the maximum of three courtships. Kendall's tau test on the four sex ratios depicted in Figure 7.6 showed a significant difference in distribution (Statistics box 7.2).

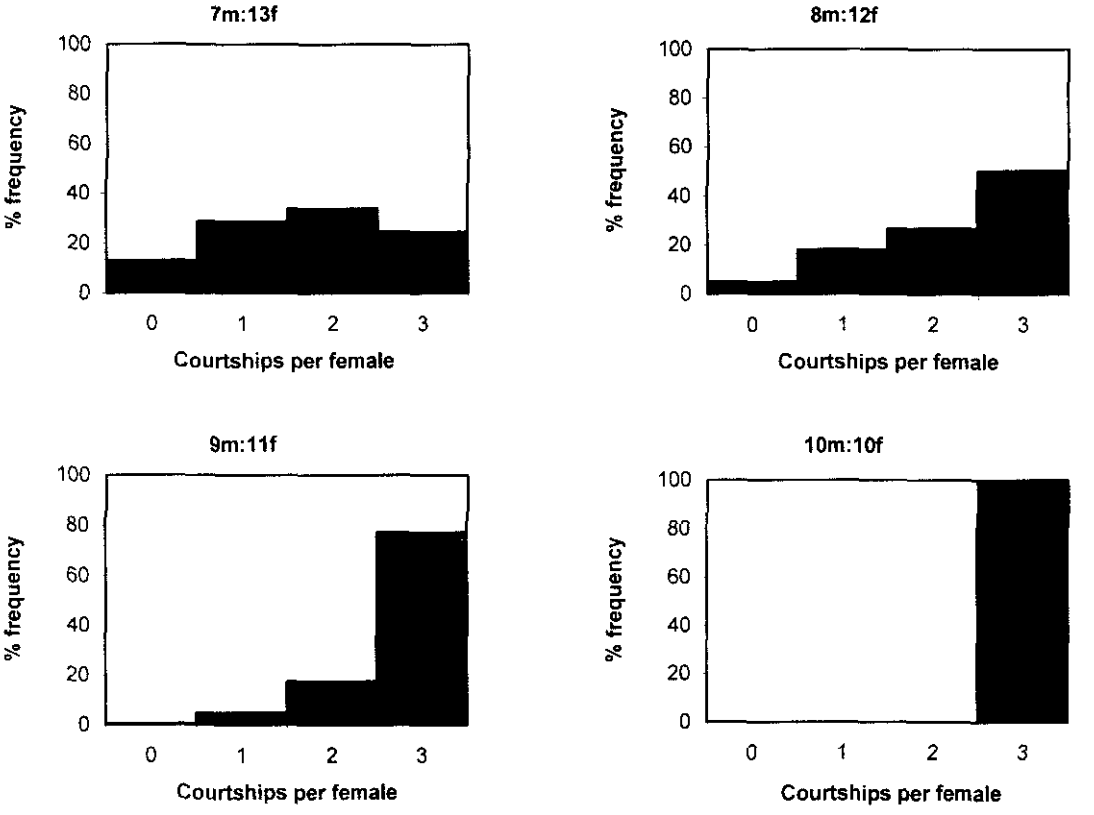


Figure 7.6. Percentage frequency distribution of *courtships per female* at four different sex ratios. Results pooled from 30 replicates (600 individuals) at each sex ratio.

Statistics box 7.2

Kendall's tau is a non-parametric test which assesses the degree of concordance and divergence between pairs of values (in this case the sex ratio and the number of *females per male*). It is suitable for ordinal data and is preferable to the Spearman rank-order correlation when there are many ties in the data.

Kendall's tau to test for a correlation between sex ratio and *females per male*: $\tau = 0.313$, $n = 1710$, standard error = 0.017, $\mathbf{p < 0.001}$.

Kendall's tau to test for a correlation between sex ratio and *courtships per female*: $\tau = 0.509$, $n = 1380$, standard error = 0.015, $\mathbf{p < 0.001}$.

It can be seen that a number of the above distributions were not normally distributed. The mean, standard deviation and variance scores presented earlier should thus be treated with some caution, in particular for the number of *courtships per female*. Nevertheless, the direction of change implied by these values is clearly valid.

Summary

1. A breeding sex ratio of unity produced a male-biased operational sex ratio by the end of the simulation. A breeding sex ratio that was weakly female-biased, on the other hand, produced a strongly female-biased OSR over time.

2. Mean *females per male* (the number of females for whom each male deposited at least one spermatophore) decreased and variance increased with an increasingly male-biased population and this was accompanied by a rise in *courting males per female* (the number of males attempting to court each female).

3. Within the female-biased populations, variance in *females per male* was reduced. As a consequence of the low incidence of interference and the limited spermatophore supply the mean number of *females per male* levelled off at about 3.5.

4. Variance in *courtships per female* (the number of encounters obtained by each female in which at least one spermatophore was deposited) occurred only in the female-biased populations.

7.4.2 Male reproductive rate

The above analyses were repeated with the spermatophore supply reduced from eight to six. The results from both simulations are presented together for ease of comparison.

The major effect of a reduction in the spermatophore supply was a shift from a male- to a female-biased operational sex ratio within a breeding population at unity (Figure 7.7). At the female-biased breeding sex ratio, the development of a completely female-biased OSR (no

sexually active males remaining) occurred more rapidly, reflecting the speed with which spermatophores were depleted.

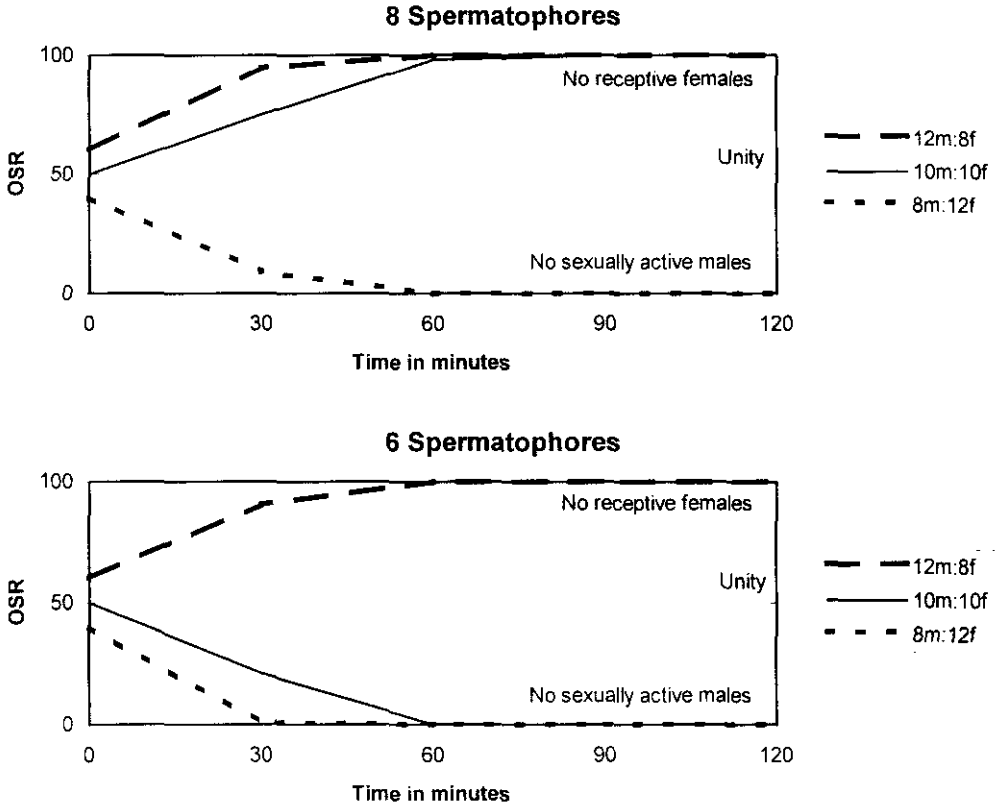


Figure 7.7. Changes in the operational sex ratio over time. Results pooled from 30 replicates (600 individuals) at each sex ratio.

The development of male- or female-biased OSRs are reflected by the means and variances in the number of *females per male* and *courtships per female*, and in the median number of *courting males per female* (Figure 7.8, A to H and Figure 7.9, A and B).

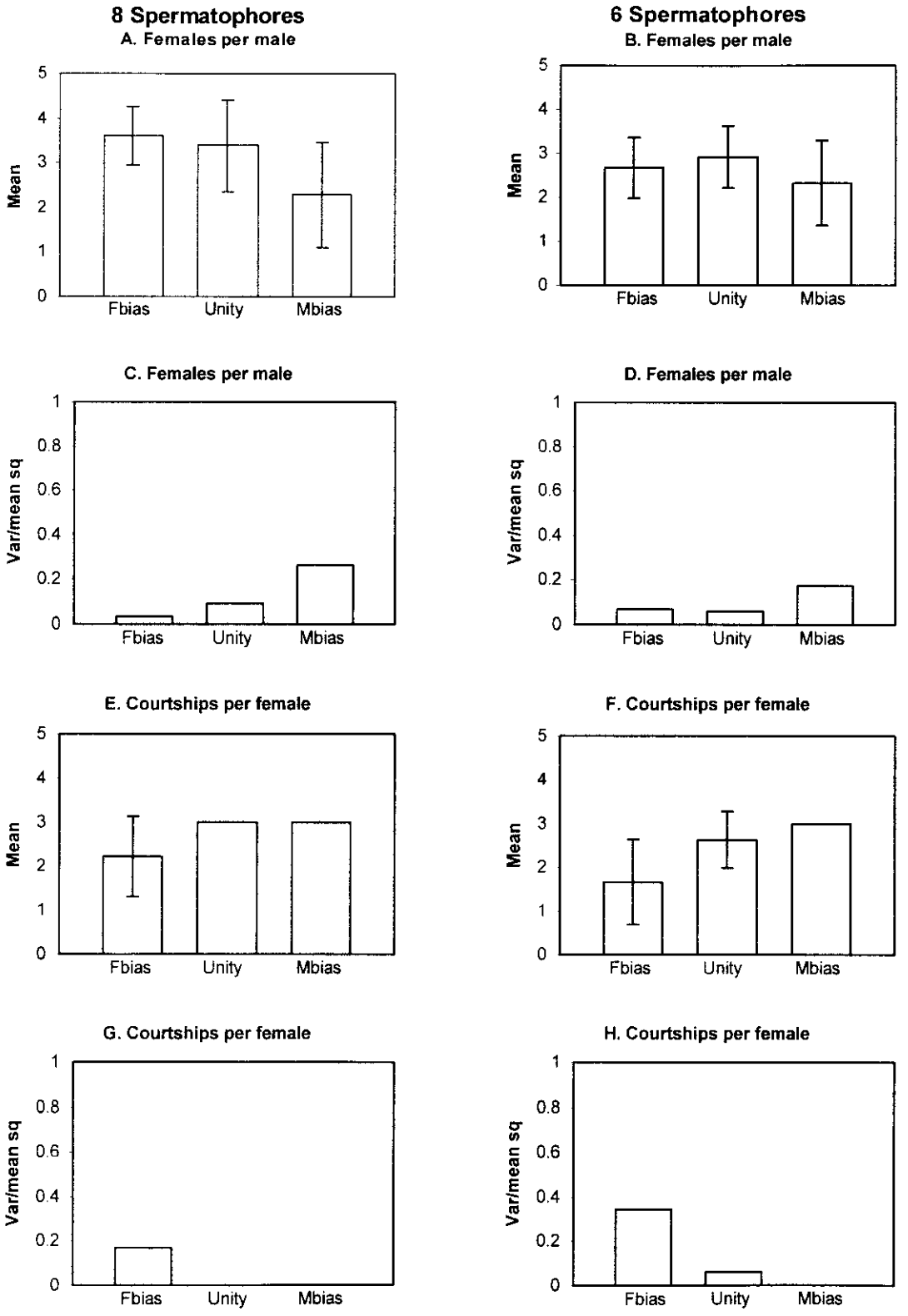


Figure 7.8. Mean (± SD) and standardised variance in *females per male* (A to D) and *courtships per female* (E to H). Fbias = 8m:12f, Unity = 10m:10f, Mbias = 12m:8f. Results pooled from 30 replicates (600 individuals) at each sex ratio.

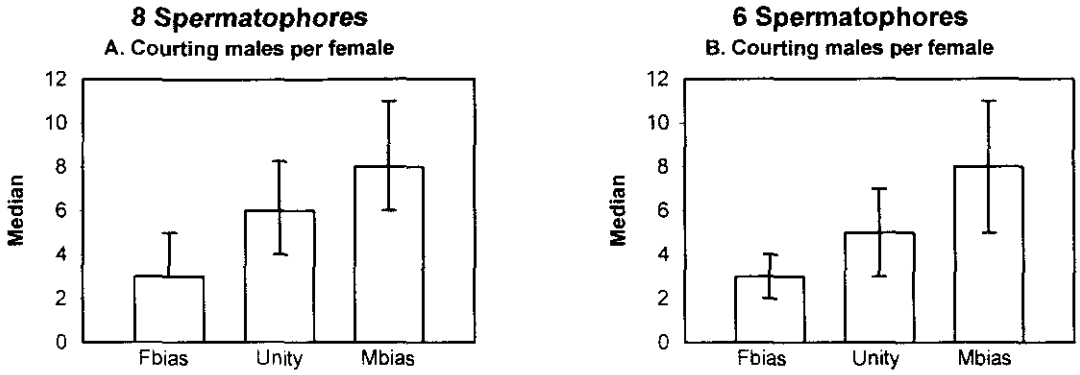


Figure 7.9. Median (± interquartile range) in *courting males per female* (A and B). Fbias = 8m:12f, Unity = 10m:10f, Mbias = 12m:8f. Results pooled from 30 replicates (600 individuals) at each sex ratio.

At the female-biased and unity sex ratios, a reduction in spermatophore supply from eight to six resulted in a reduction in the mean number of *females per male* (Figure 7.8, A and B). The relatively low levels of interference at these sex ratios (Figure 7.9) allowed males to allocate their six spermatophores between two or three females only. Even within the male-biased population, there was a reduction in the variance in *females per male* with a spermatophore supply of six (Figure 7.8D). Despite frequent interference, males would rarely have been able to distribute six spermatophores between four or more females.

The difference between the two simulations was particularly marked in the case of *courtships per female*. At a breeding sex ratio of unity, six spermatophores per male were not sufficient to ensure that all females obtained three matings and the result was a female-biased OSR and variance in the number of *courtships per female*. There was also a considerable decrease in the mean and an increase in the variance of *courtships per female* at the female-biased sex ratio.

The number of *courting males per female* differed significantly between six and eight spermatophores per male at the female-biased and unity sex ratios (the median test, Statistics box 7.3). At these sex ratios, males with only six spermatophores had fewer opportunities to interfere. At the male-biased sex ratio, six spermatophores were sufficient to allow competition between males.

The frequency distributions of *females per male* (Figure 7.10) and *courtships per female* (Figure 7.11) give a more detailed picture of the changes taking place at different sex ratios.

Statistics box 7.3

The median test (Siegel and Castellan 1988) to establish whether the median number of *courting males per female* was significantly different in simulations with a spermatophore supply of eight or six.

Female-bias: $X^2 = 12.23$, df = 1, m = 360, n = 360, $\mathbf{p < 0.001}$. Unity: $X^2 = 13.52$, df = 1, m = 300, n = 300, $\mathbf{p < 0.001}$. Male-bias: $X^2 = 1.65$, df = 1, m = 240, n = 240, $p > 0.1$.

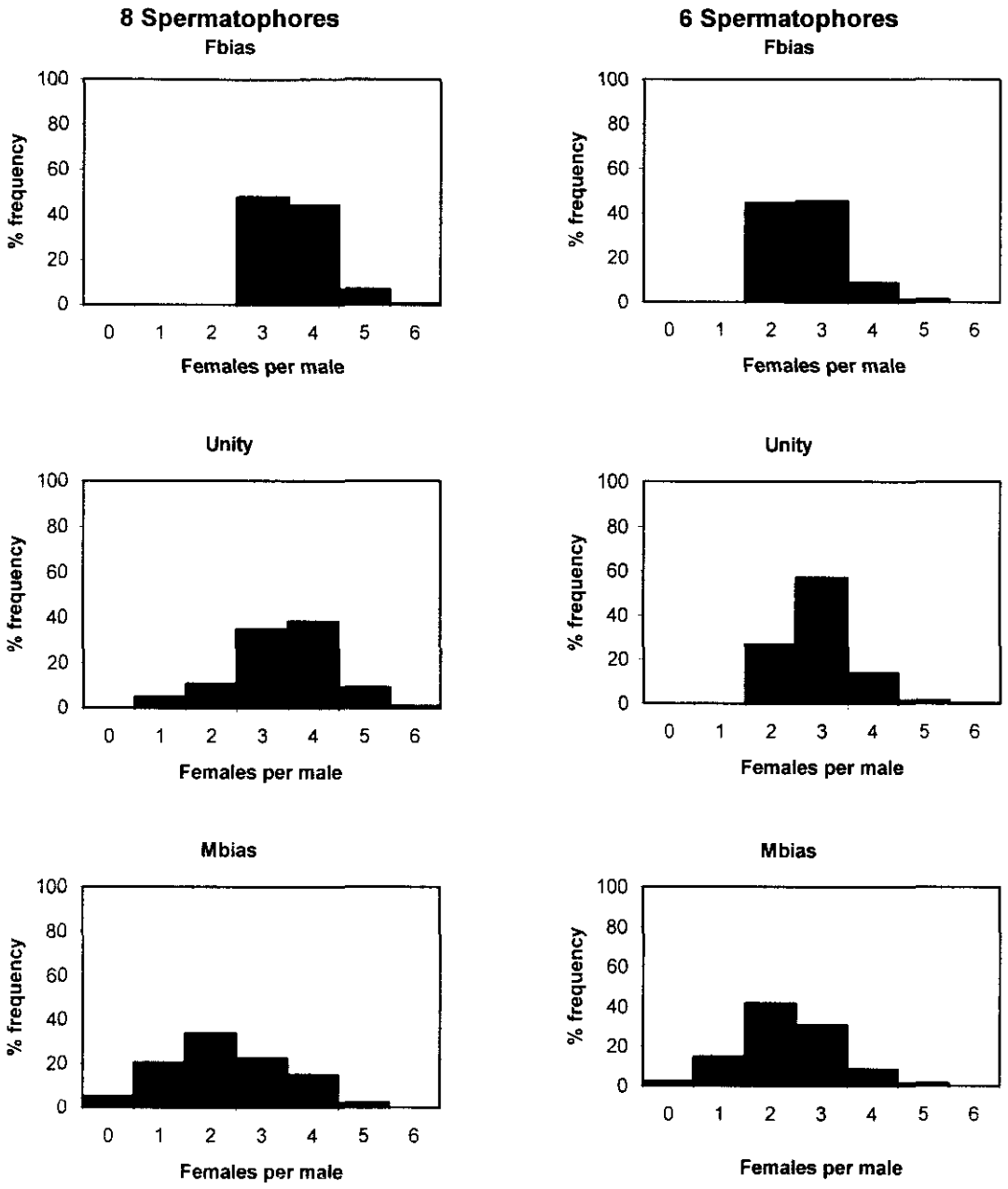


Figure 7.10. Percentage frequency distribution of *females per male*. Fbias = 8m:12f, Unity = 10m:10f, Mbias = 12m:8f. Results pooled from 30 replicates (600 individuals) at each sex ratio.

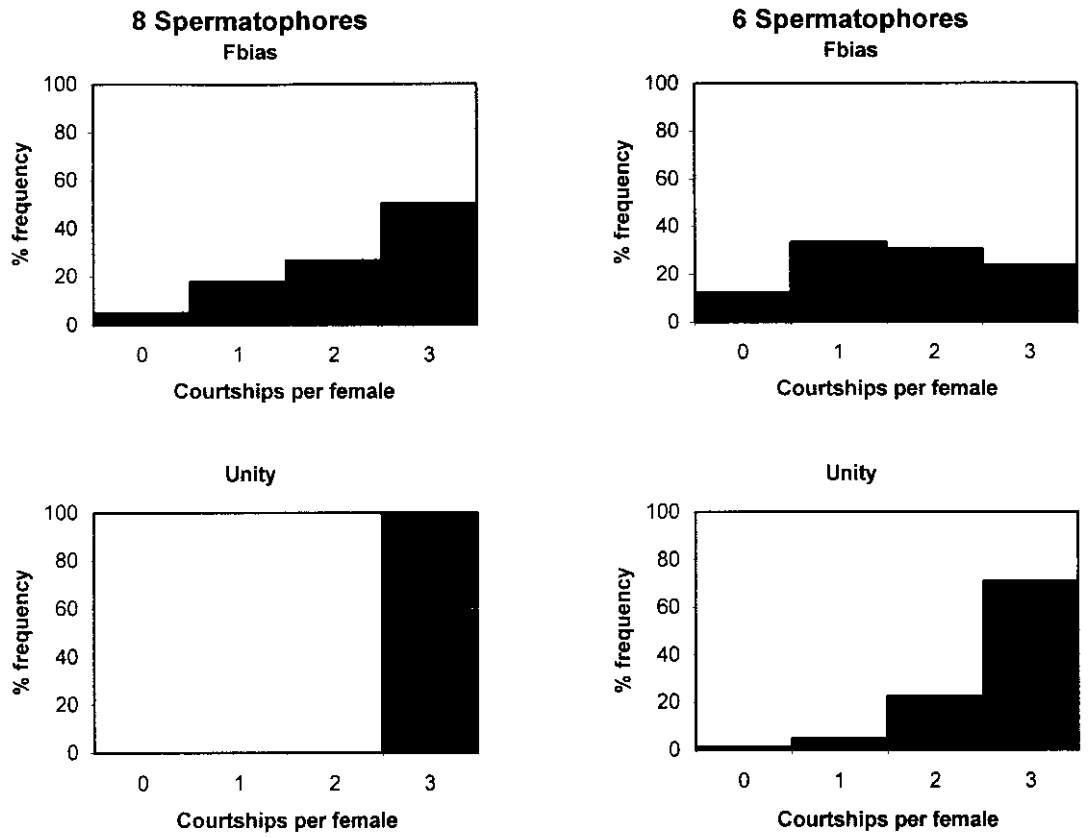


Figure 7.11. Percentage frequency distribution of *courtships per female*. Fbias = 8m:12f, Unity = 10m:10f, Mbias = 12m:8f. Results pooled from 30 replicates (600 individuals) at each sex ratio.

The differences in the frequency distribution of *females per male* between the two simulations were significant for both unity and female-biased sex ratios but not for the male-biased sex ratio (Statistics box 7.4A). Kolmogorov-Smirnov tests also revealed significant differences between distributions of *courtships per female* at both unity and female-biased sex ratios (Statistics box 7.4B).

Statistics box 7.4

The Kolmogorov-Smirnov two sample test to compare the distribution of *females per male* and *courtships per female* between simulations with a maximum spermatophore supply of eight or six.

A. *Females per male*. Female-bias: $D_{mn} = 0.45$, m = 240, n = 240, **$p < 0.001$**. Unity: $D_{mn} = 0.32$, m = 300, n = 300, **$p < 0.001$**. Male-bias: $D_{mn} = 0.08$, m = 360, n = 360, $p > 0.1$.

B. *Courtships per female*. Female-bias: $D_{mn} = 0.26$, m = 360, n = 360, **$p < 0.001$**. Unity: $D_{mn} = 0.29$, m = 300, n = 300, **$p < 0.001$**.

Summary

1. The reduction in spermatophore supply produced a switch from the development of a male-biased OSR to a female-biased OSR within a breeding sex ratio at unity.

2. At female-biased and unity sex ratios, the reduction in spermatophore supply caused a decrease in mean *females per male* (the number of females for whom each male deposited at least one spermatophore) as males distributed their six spermatophores between just two or three females. Mean *courtships per female* (the number of encounters obtained by each female in which at least one spermatophore was deposited) decreased and variance increased considerably at these sex ratios. The male-biased sex ratio was less affected by the change in reproductive rate.

3. A spermatophore supply of six resulted in fewer *courting males per female* (the number of males attempting to court each female) at the female-biased and unity sex ratios.

7.4.3 The duration of the arrival period

The two populations were compared after the arrival of all 40 individuals (day one of the synchronous population compared with day four of the asynchronous population). In seven out of the eight simulations, the final operational sex ratio contained no sexually receptive females (all females having used up three courtship opportunities). Even the female-biased asynchronous breeding population had reached a male-biased OSR by the end of the fourth day and it was only the *female-biased synchronous* population which developed a female-biased OSR.

The mean and standardised variance scores for the number of *females per male* and *courtships per female* and the median number of *courting males per female* are shown in Figure 7.12 and Figure 7.13.

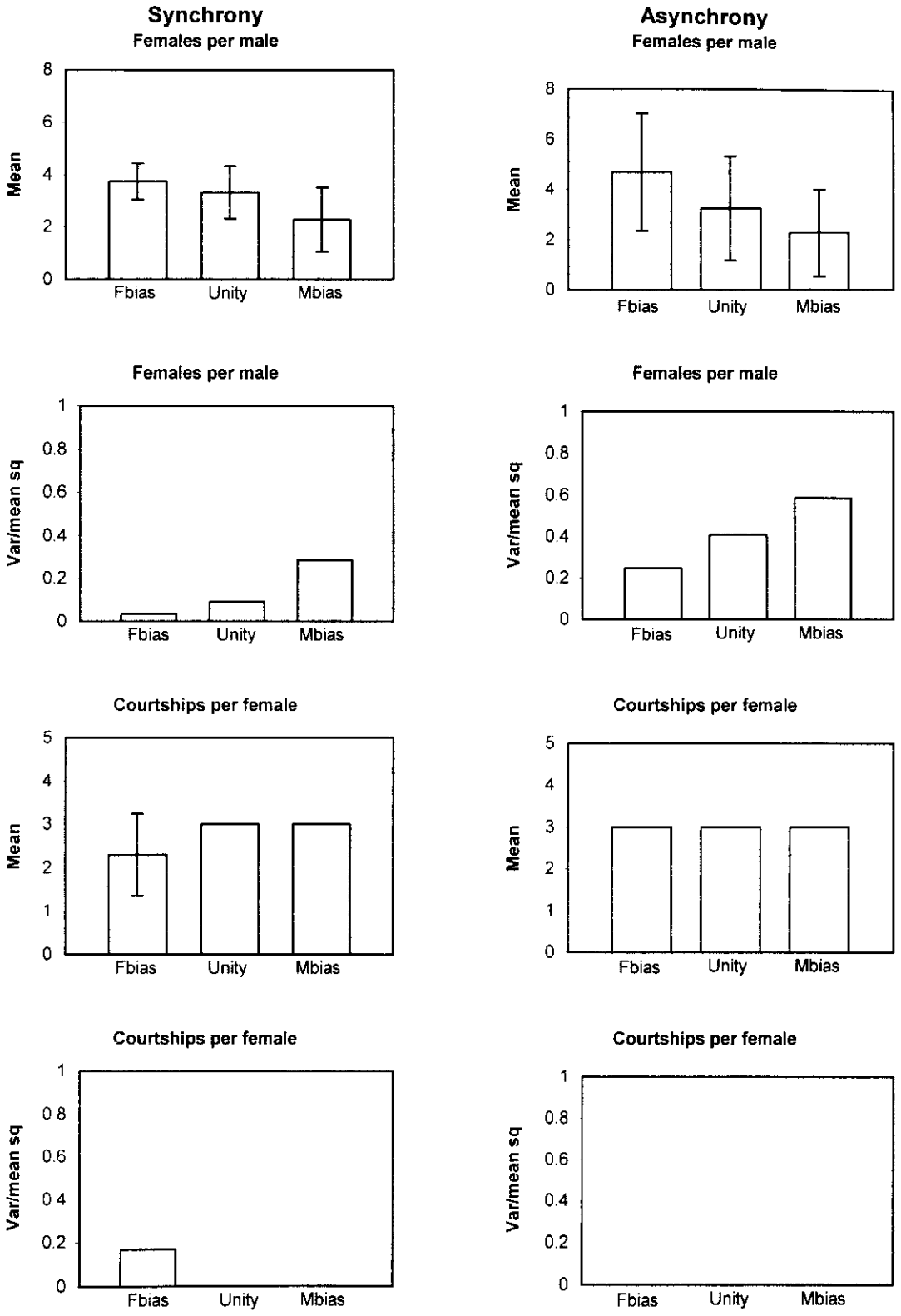


Figure 7.12. Synchronous and asynchronous populations: mean (±SD) and standardised variance in *females per male* and *courtships per female* at three sex ratios. Fbias = 16m:24f, Unity = 20m:20f, Mbias = 24m:16f. Results pooled from 30 replicates (1200 individuals) at each sex ratio.

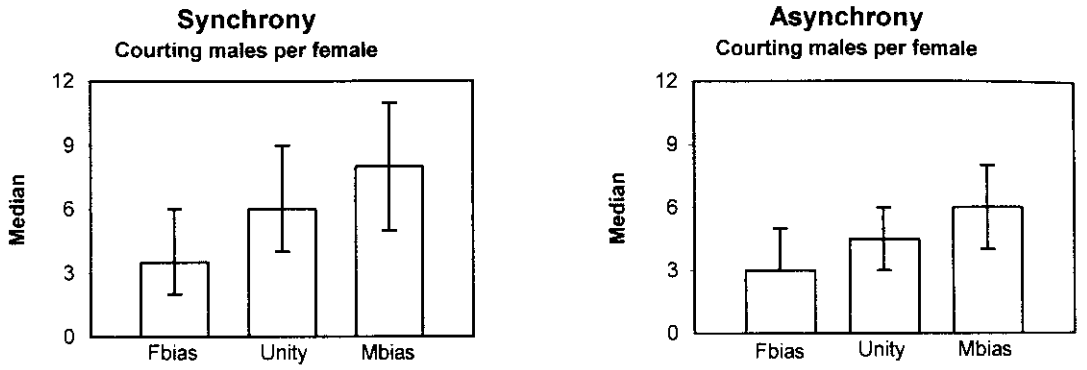


Figure 7.13. Synchronous and asynchronous populations: median (± interquartile range) in *courting males per female* at three sex ratios. Fbias = 16m:24f, Unity = 20m:20f, Mbias = 24m:16f. Results pooled from 30 replicates (1200 individuals) at each sex ratio.

The variance in the number of *females per male* increased at *all sex ratios* when the population was introduced asynchronously. However a difference in the number of *courtships per female* was seen only between the *female-biased* breeding sex ratios, reflecting the differences in the OSR mentioned above. At the female-biased sex ratio, synchronous arrival resulted in the depletion of male spermatophore supply before female courtship opportunities were used up, a female-biased OSR and variance in *courtships per female*. Asynchronous introduction of the population, with the associated ability of males to renew their spermatophore supply, resulted in female mating opportunities being used up before male spermatophore supply, a male-biased OSR and thus no variance in the number of *courtships per female*.

On the other hand, median values of *courting males per female* were significantly higher for the synchronous population at male-biased and even sex ratios but not at the female-biased sex ratio (the median test, Statistics box 7.5).

Statistics box 7.5

The median test (Siegel and Castellan 1988) to establish whether the median number of *courting males per female* was significantly different in simulations with synchronous and asynchronous arrival.

Female-bias: $X^2 = 0.56$, df = 1, m = 720, n = 720, $p > 0.7$. Unity: $X^2 = 69.9$, df = 1, m = 600, n = 600, $\mathbf{p < 0.001}$. Male-bias: $X^2 = 41.07$, df = 1, m = 480, n = 480, $\mathbf{p < 0.001}$.

The frequency distributions of *females per male* are shown in Figure 7.14. Since variance in the number of *courtships per female* only existed within one (female-biased, synchronous) population, I have not presented the frequency distributions.

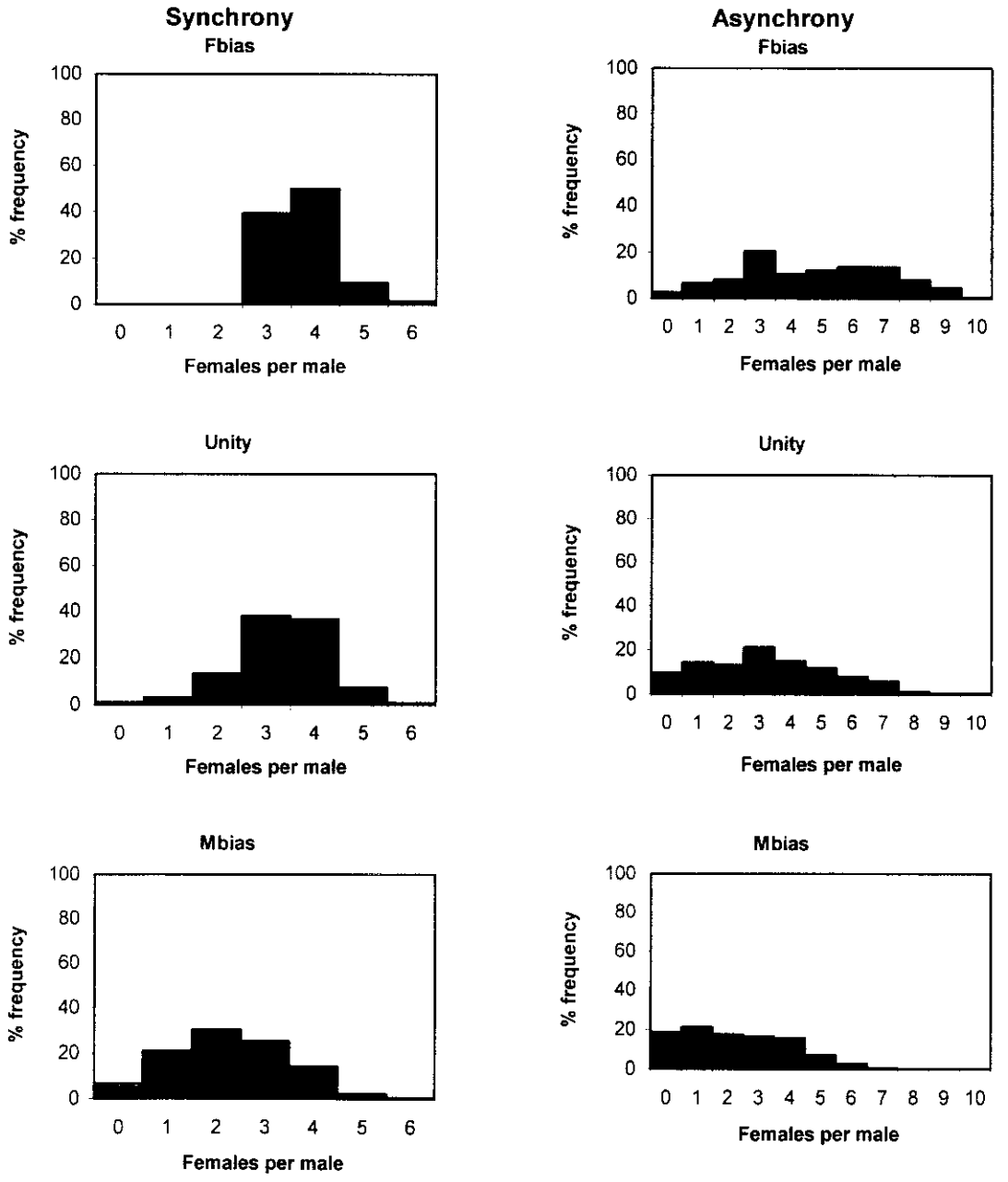


Figure 7.14. Synchronous and asynchronous populations: percentage frequency distribution of *females per male* at the three sex ratios: Fbias = 16m:24f, Unity = 20m:20f, Mbias = 24m:16f. Results pooled from 30 replicates (1200 individuals) at each sex ratio.

The increase in variance in the asynchronous population is very clearly illustrated by Figure 7.14, with the maximum number of *females per male* rising from six in the synchronous

population to 10 in the asynchronous population. The differences in distribution were significant at all three sex ratios (Kolmogorov-Smirnov, Statistics box 7.6). There was also a marked increase in the number of males failing to mate (scoring zero) as the sex ratio changed from female- to male-biased and the population changed from synchronous to asynchronous. Almost 20% of males failed to mate in the male-biased asynchronous population compared with none in the female-biased synchronous population.

Summary

1. A comparison between synchronous and asynchronous populations after the arrival of all 40 individuals showed greater variance in *females per male* (the number of females for whom each male deposited at least one spermatophore) within the asynchronous populations at all sex ratios. A female-biased operational sex ratio and variance in *courtships per female* (the number of encounters obtained by each female in which at least one spermatophore was deposited) occurred only within the female-biased *synchronous* population. At unity and male-biased sex ratios, *courting males per female* (the number of males attempting to court each female) was higher in the synchronous population.

7.4.4 Arrival of the asynchronous population

The four-day arrival period of the *asynchronous* population was analysed in more detail. The *operational sex ratio* was male-biased at all sex ratios after four days but remained female-biased within the female-biased breeding population until day three (Table 7.2) and this was reflected by variance in *courtships per female* (Table 7.3).

Statistics box 7.6

The Kolmogorov-Smirnov two sample test to compare the distribution of *females per male* between simulations with synchronous and asynchronous arrival.

Female-bias: $D_{mn} = 0.4$, m = 480, n = 480, $\mathbf{p < 0.001}$. Unity: $D_{mn} = 0.19$, m = 600, n = 600, $\mathbf{p < 0.001}$. Male-bias: $D_{mn} = 0.12$, m = 720, n = 720, $\mathbf{p < 0.001}$.

	Fbias	Unity	Mbias
Day one	9	100	100
Day two	33	100	100
Day three	100	100	100
Day four	100	100	100

Table 7.2. Asynchronous population: the operational sex ratio at the end of each day. 100: no more receptive females available, 50: unity, 0: no more sexually active males. Population size: day one = 10, day two = 20, day three = 30, day four = 40. Results pooled from 30 replicates at each sex ratio.

	Day one (n = 10)	Day two (n = 20)	Day three (n = 30)	Day four (n = 40)
$\bar{x}$	2.22	2.93	3	3
SD	0.82	0.3	0	0
$\sigma^2/\bar{x}^2$	0.14	0.01	0	0

Table 7.3. Female-biased asynchronous population: changes in the mean, standard deviation and standardised variance of *courtships per female* over the four-day arrival period. n: population size each day. Results pooled from 30 replicates at each sex ratio.

At the female-biased breeding sex ratio, it was not until the third day that sufficient numbers of males had completed the refractory period and returned to the pool of sexually active individuals to cause a shift to a male-biased OSR.

In section 7.4.3 above, it was shown that the variance in the number of *females per male* was higher in the asynchronous than in the synchronous population. This was not surprising considering that within the asynchronous population, some males spent longer in the pond than others, renewed their spermatophore supply and had the opportunity to court a greater number of females. Figure 7.15 illustrates the gradual change in variance over the four-day arrival period.

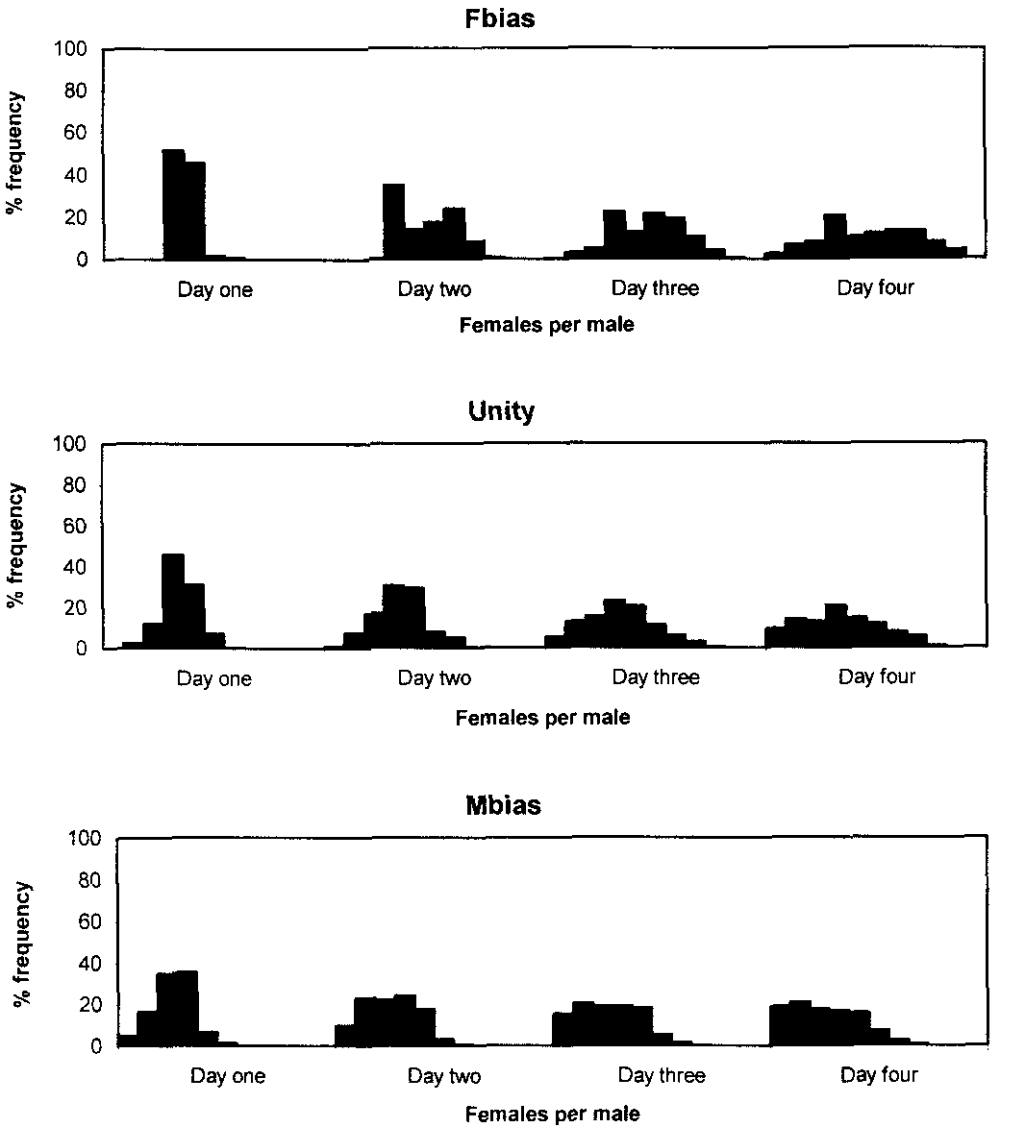


Figure 7.15. Asynchronous population: percentage frequency distribution in *females per male* during the four-day arrival period. Population size: day one = 10, day two = 20, day three = 30, day four = 40. Results pooled from 30 replicates at each sex ratio.

There are no appropriate statistical tests for this data set, which contains a combination of matched and unmatched data, but the figure helps to visualise the changes taking place during arrival.

The increase in variance in the number of *females per male* during the arrival period was expected to be accompanied by an increase in the amount of interference between males. However, the median and interquartile range values for *courting males per female* remained remarkably constant (Table 7.3).

		Day one (n = 10)	Day two (n = 20)	Day three (n = 30)	Day four (n = 40)
Fbias	Median	3	3	3	3
	1st quartile	2	3	3	3
	3rd quartile	4	5	5	5
Unity	Median	5	5	5	4.5
	1st quartile	4	3	3	3
	3rd quartile	7	7	6	6
Mbias	Median	6	6.5	6	6
	1st quartile	5	5	4	4
	3rd quartile	9	9	8	8

Table 7.4. Asynchronous population: changes in the median and interquartile range of *courting males per female* over the four-day arrival period. n: population size each day. Results pooled from 30 replicates at each sex ratio.

The most likely explanation is that within the model (and in reality, Chapter three), males spend a considerable time following unreceptive females. During the introduction of each asynchronous population, the ratio of receptive to unreceptive females decreases but the sex ratio remains the same. *Within* each sex ratio, therefore, the variance in *females per male* arises as a result of differences between males in their encounter rate of receptive females rather than in increasing amounts of competition between males.

Figure 7.15 gives an overview of changes in the frequency distribution of the number of *females per male* over the four-day arrival period but combines individuals who were present in the pond for varying periods of time. The *relative* success of individuals, arriving on different days can be assessed by comparing the percentage frequency distribution of *females per male* for each newly-arriving group of males on their first night only (Figure 7.16). Day one refers to those males arriving on day one, day two to males arriving on day two and so on.

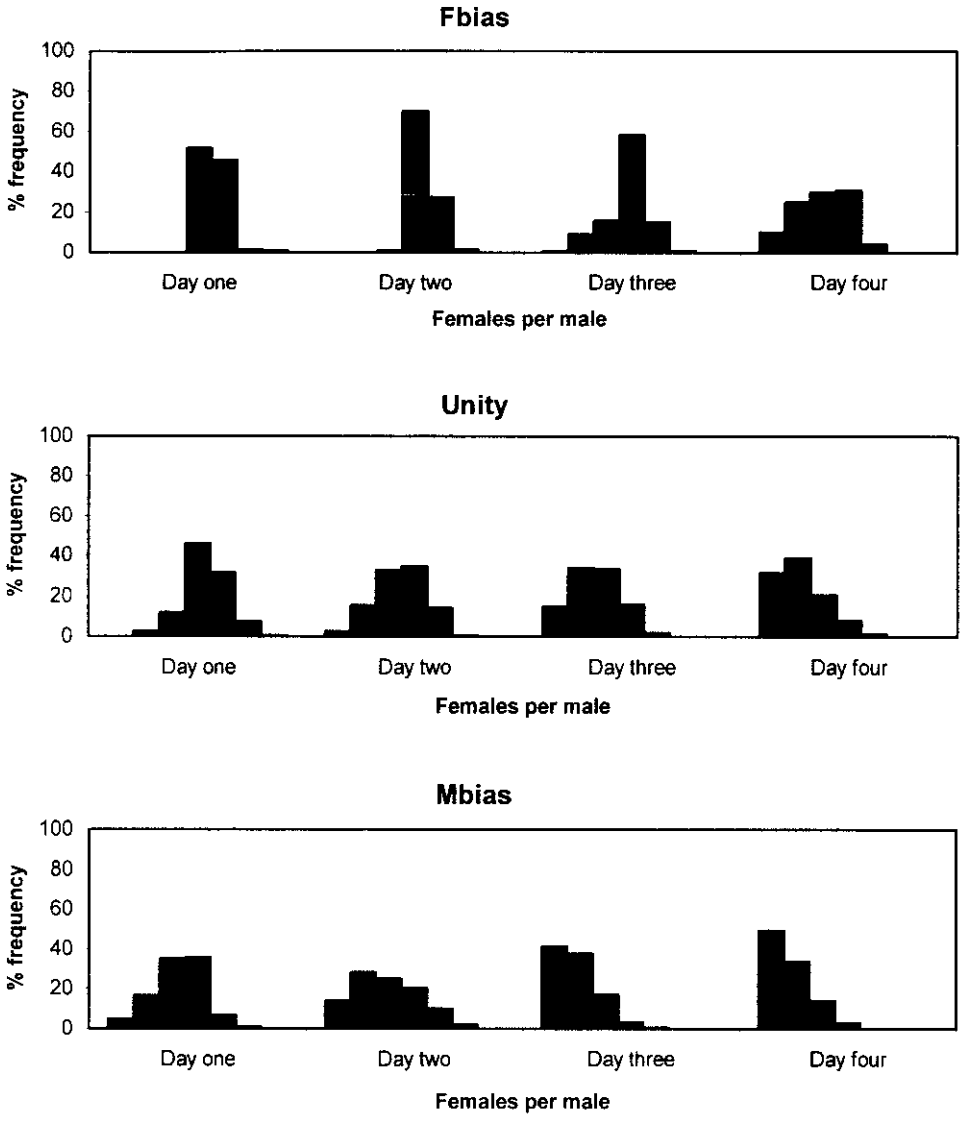


Figure 7.16. Asynchronous population: percentage frequency distribution in *females per male* for each new group of males on their first night in the pond. Population size: day one = 10, day two = 20, day three = 30, day four = 40. Results pooled from 30 replicates at each sex ratio.

There was a significant negative correlation between the day of arrival and *females per male* at all sex ratios (Kendall's tau, Statistics box 7.7). Males arriving later obtained fewer females on their first night and were more likely not to mate at all.

Summary

1. A more detailed analysis of the female-biased asynchronous population revealed a female-biased operational sex ratio and variance in *courtships per female* (the number of encounters obtained by each female in which at least one spermatophore was deposited) at the end of the first two days.
2. The increase in variance in *females per male* (the number of females for whom each male deposited at least one spermatophore) *within* each asynchronous population was considered to be the result of changes in the encounter rate of receptive females rather than direct competition through interference.
3. The relative mating success of each newly-arriving group of males decreased over time.

7.4.5 Summary of results from Chapter seven

Breeding sex ratio

1. A breeding sex ratio of unity produced a male-biased operational sex ratio by the end of the simulation. A breeding sex ratio that was weakly female-biased, on the other hand, produced a strongly female-biased operational sex ratio over time.
2. The mean number of *females per male* decreased and variance increased with an increasingly male-biased population and this was accompanied by a rise in the number of males courting each female.

Statistics box 7.7

Kendall's tau to test for a correlation between day of arrival and *females per male*. Female-bias: $\tau = -0.52$, $n = 480$, standard error = 0.027, $\mathbf{p < 0.001}$. Unity: $\tau = -0.56$, $n = 600$, standard error = 0.022, $\mathbf{p < 0.001}$. Male-bias: $\tau = -0.449$, $n = 720$, standard error = 0.023, $\mathbf{p < 0.001}$.

3. Within the female-biased populations, variance in *females per male* was reduced. As a consequence of the low incidence of interference and the limited spermatophore supply, the mean number of *females per male* levelled off at about 3.5.

4. Variance in the number of *courtships per female* occurred only in the female-biased populations.

Reproductive rate

1. The reduction in spermatophore supply produced a switch from the development of a male-biased OSR to a female-biased OSR within a breeding sex ratio at unity.

2. At female-biased and unity sex ratios, the reduction in spermatophore supply caused a decrease in mean *females per male* as males distributed their six spermatophores between just two or three females. The mean number of *courtships per female* decreased and variance increased considerably at these sex ratios. The male-biased sex ratio was less affected by the change in reproductive rate.

3. A spermatophore supply of six resulted in fewer *courting males per female* at the female-biased and unity sex ratios.

Synchronous and asynchronous arrival

1. A comparison between synchronous and asynchronous populations after the arrival of all 40 individuals showed greater variance in *females per male* within the asynchronous populations at all sex ratios. A female-biased OSR and variance in *courtships per female* occurred only within the female-biased *synchronous* population. At unity and male-biased sex ratios the number of *courting males per female* was higher in the synchronous population.

2. A more detailed analysis of the female-biased asynchronous population revealed a female-biased OSR and variance in *courtships per female* at the end of the first two days.

3. The increase in variance in the number of *females per male* within each asynchronous population was considered to be the result of changes in the encounter rate of receptive females rather than direct competition through interference.

4. The relative mating success of each newly-arriving group of males decreased over time.

7.5 Discussion

7.5.1 Breeding sex ratio and reproductive rate

The aim of this model was to simulate the mating dynamics of smooth newts under a variety of conditions at the start of the season and to establish the effect these had on individual mating success and the potential for sexual selection. Once again, I wish to emphasise that it was necessary to define mating success in terms of courtships with deposition because there is insufficient understanding of the mechanisms governing pick-up to incorporate insemination success into the model.

Within the single-night simulations, the results were largely determined by the relative rate at which the mating opportunities of each sex were depleted and thus the speed at which the operational sex ratio became increasingly male- or female-biased. The model confirmed the expectation that a *male-biased* breeding sex ratio should produce an increasingly male-biased operational sex ratio, increased competition between males and greater variance in male mating success. Likewise, the *female-biased* population developed an increasingly female-biased OSR (as male spermatophore supply was used up), exhibited variance in the number of *courtships per female* and theoretically created the opportunity for competition between females.

The relationship between interference and mating success was less straightforward. Within the model, successful males were those which encountered receptive females and had remaining spermatophores for courtship. Interference played a dual role in this respect. It

resulted either in the termination of courtship before deposition took place (representing a lost courtship opportunity for the courting male) or in an encounter with just one or two depositions per male, providing the potential for further courtships. Although this paradox arose as a result of the model structure, it has again emphasised the need to understand the way in which males distribute spermatophores between females in the wild and the effect this has on their breeding success. As discussed in Chapter four, in a semi-natural situation males rarely deposit more than one or two spermatophores per encounter. It would be interesting to run another model simulation with a reduction in the maximum number of depositions per encounter.

There is a substantial collection of laboratory and field-based studies (insects and fish being particularly well represented) which demonstrate the relationship between sex ratio and mating success. Many studies confirm that a sex ratio biased towards the competing sex produces greater competition for mates and greater variance in mating success (for example Colwell and Oring 1988; Carroll and Salamon 1995; Grant et al. 1995; Kvarnemo et al. 1995). Changes in the sex ratio, however, may lead to complex responses. Ward (1988) manipulated the sex ratio in natural populations of threespine sticklebacks (*Gasterosteus aculeatus*). Low reproductive success occurred in male-biased pools since many males failed to complete nesting, but male mating success was also reduced under female-biased sex ratios (in comparison with unbiased pools) as a result of increased nest raiding by competing females. It should also be stressed that the relationship between the OSR and the intensity of competition will depend on the costs and benefits of each particular competitive behaviour, factors which may vary between species and populations (Clutton-Brock and Parker 1992).

As mentioned above, the model results reflected the relative rates at which the mating opportunities of each sex were used up. This was clearly illustrated by the simulations in which male spematophore supply was reduced from eight to six. The population at unity

developed a female-biased (as opposed to a male-biased) operational sex ratio. Females still had courtship opportunities after males had deposited all six spermatophores and there was variance in the number of *courtships per female*. The female-biased population experienced an increase in the variance in *courtships per female* and a decrease in the number of *females per male*. The male-biased population remained relatively unaffected since the high rate of interference ensured that six spermatophores were sufficient to complete female mating opportunities. An even greater reduction in spermatophore supply would be necessary to produce an equivalent effect in a male-biased sex ratio.

The prediction that a reduction in the spermatophore supply of males should reduce the ratio of sexually active males to receptive females was thus substantiated. In the future, the model could be used to vary the number of courtships engaged in by each female and the duration of the period needed for sperm replenishment, additional factors which affect reproductive rate in smooth newts.

In a number of species, the relative reproductive rates of males and females have been shown to vary naturally, in response to fluctuations in temperature and food supply (Bush 1993; Kvarnemo 1994; Ahnesjö 1995). In sand gobies (*Pomatoschistus minutus*), for example, male reproductive rate (determined by paternal care of egg clutches) is more strongly influenced by temperature than female reproductive rate (determined by egg development in the ovary). As a result, the operational sex ratio shifts from unity to increasingly male-biased as water temperatures rise over the season (Kvarnemo 1994). Temperature also affects the rate at which male midwife toads (*Alytes muletensis*) can brood eggs, while the rate at which females produce new clutches is determined by the food supply. As temperature and food supply vary over the long season, relative reproductive rates are altered accordingly and the sex with the higher reproductive rate competes for mates (Bush 1993).

In smooth newts, reproductive rate is determined by spermatophore production in males and the multiple mating patterns of females. Environmental control of spermatophore production has not been demonstrated experimentally but observation of smooth newts indicates that fewer spermatophores are produced on cold days (pers. obs.; Waights, pers. comm.), presumably because male motivation or the physiological ability to produce spermatophores is reduced. In the light of the model findings, it is reasonable to suggest that a reduction in spermatophore production during the first cold weeks of the season would have a significant effect on the direction of competition for mates. Although the mechanisms which determine female mating patterns over the season have not yet been determined, egg-laying is likely to play an important role and this has also been shown to be influenced by temperature (Hosie 1992).

7.5.2 Synchronous and asynchronous arrival

The model results discussed so far have dealt with closed populations in which all potential courtships were completed before the arrival of any new individuals. This is an unrealistic scenario for the smooth newt, which has a long immigration period. Hence the last two simulations compared the arrival of 40 individuals over one and four days (synchronous and asynchronous arrival). The asynchronous arrival pattern incorporated the critical difference between male and female courtship activity at the beginning of the season: males had the opportunity to replenish their spermatophore supplies following a 24 hour rest period whereas females remained unreceptive after completing three courtships.

As predicted, variance in male mating success was greater in asynchronous than synchronous populations. Males which arrived first had the opportunity to replenish their spermatohore supply and court a greater number of females. In addition, males arriving later experienced a relatively more competitive environment on their first night in the pond. It was particularly significant that, even when each newly-arrived population was *female-biased*, the

operational sex ratio soon switched to a *male bias* as more males renewed their spermatophore supply. I suggest that this is likely to be a true reflection of a natural population and predict that under most circumstances, even a female-biased population will soon develop a male-biased OSR. The degree of bias will fluctuate as different numbers of males and females arrive throughout the immigration period. A female-biased OSR can be envisaged only if a highly female-biased population arrives simultaneously (see Chapter six) and over a short period of time. In a model which investigated the relative importance of the sex ratio, the spatial distribution of females and asynchrony in female receptivity, Ims (1988) found the degree of asynchrony to be the most influential factor on variance in male mating success.

As discussed in Chapter six, males would appear to benefit from arriving before females at the breeding pond. Four factors were proposed to account for the lack of consistency in early male arrival within smooth newt populations: multiple mating by females, female-biased populations, costs incurred by inhabiting a pond environment in the early spring and benefits associated with mating later in the season. According to the model presented in this chapter, neither multiple mating by females nor female-biased populations counteract the increase in competition faced by late arriving males.

Although the variance in mating success was greater in the asynchronous population, the number of *courting males per female* was higher in the synchronous population. Tejedo (1988) reported that fighting between male *Bufo calamita* is rarely observed except in high density populations in which arrival is relatively synchronous (two to three weeks compared with the more usual eight weeks). He suggested that during asynchronous arrival, aggressive males encounter more single males than pairs, reducing the opportunity for direct competition.

A similar situation may be envisaged for the smooth newt in which competition between males in the asynchronous population is increasingly characterised by the ability to find receptive females rather than by sexual interference. According to Schwagmeyer and

Woontner (1985), the encounter rate of receptive females is particularly important in species (such as smooth newts) in which the time spent searching is long in comparison with time spent in courtship and mating. Under these circumstances, the extent to which male smooth newts can recognise receptive females is clearly important. Males are more attracted to unmated, and mated but unfamiliar females, both of which have a greater probability of being receptive (Chapter five). On the other hand, males not only spend a large proportion of time chasing unreceptive females but are easily distracted by other males (Chapters three and four).

According to results from the model, the operational sex ratio and the degree of competition for mates in the smooth newt will vary both within and between breeding seasons in response to variation in the breeding sex ratio, reproductive rate and arrival patterns. Natural variation in the degree and direction of bias in the OSR over a variety of temporal scales has been widely reported (Sullivan 1986; Halliday and Tejedo 1995; for review see Kvarnemo and Ahnesjö 1996). For example, the OSR and the variance in mating success have been shown to fluctuate in response to variation in reproductive rate (Kvarnemo 1994), the distribution and quality of food (Lawrence 1986; Gwynne 1990; Krupa and Sih 1993) and access to nest sites (Davies et al. 1995), factors which are frequently under the control of stochastic events, such as climatic fluctuations.

In many species, these short-term fluctuations in the OSR are associated with a plasticity in mating behaviour (Emlen and Oring 1977; Fricke 1980; Berven 1981; Garstka and Crews 1985; Lawrence 1986; Krupa and Sih 1993; Berglund 1994; Davies et al. 1995). The overall OSR of Japanese medaka fish (*Oryzias latipes*) is male-biased but males take temporary opportunities to exercise their preference for large females when the local OSR reaches unity (Grant et al. 1995). Male milkweed beetles (*Tetraopes tetraophthalmus*) compete for females under male-biased conditions with a resultant large-male advantage, but exhibit a preference for large females under female-biased conditions (Lawrence 1986). Among anurans, Höglund

(1989) showed that a large male advantage or size assortative mating occurs at male-biased sex ratios (depending on the length of time available for amplexus) but that mating is random with respect to size when the sex ratio is at unity.

Male-male competition, female-female competition, male preference for large females and female preference for high crested males have all been described in the smooth newt (Verrell 1982b; 1984a; Green 1991a; Hosie 1992; Waights 1996) and I suggest that these result from a plasticity in mating behaviour which allows adaptation to changing and temporary local conditions. For example, under a female-biased OSR, females may compete through interference (Waights 1996) and males may express their preference for large females (Verrell 1986a). When the OSR becomes male-biased, male interference will occur and the potential for female choice exists. Later in the season, females may choose higher-crested males (although the problems of expressing such a preference during courtships with interference has been discussed in Chapter five).

7.5.3 The operational sex ratio and sexual selection

The model has shown that, at least during the first part of the season, there is likely to be variance in male mating success in the smooth newt. Sexual selection, however, can only act on variance in mating success if there is a *causal relationship* between individual success and a phenotypic trait or suite of characters (see also Chapter four). If not, the distribution of successful matings among individuals is a matter of chance or environmental variation (Sutherland 1985a; Hubbell and Johnson 1987; 1987; Clutton-Brock 1988; Halliday and Tejedo 1995). This was expressed particularly well by McVey (1988), 'If there are no phenotypic characteristics that influence reproductive success, the 'opportunity' for sexual selection is simply the opportunity for genetic drift'. In order to assess the effect of variance in mating success on sexual selection, it is therefore crucial to ascertain the extent to which such

variation is caused by phenotypic differences, by chance or by environmental variation (Clutton-Brock 1988).

What is the influence of phenotype on mating success in smooth newt? Male size does not appear to be linked to the ability to compete for females through sexual interference (experiment two, Chapter five), but there is no reason to believe that correlations between other morphological or behavioural characteristics and mating success do not exist (see Chapter four, for discussion on the relationship between crest height and mating success). For example, there is considerable variation among males in spermatophore production both in the short- and the long-term (Waights 1998) and males may vary in their ability to detect receptive females (experiment one, Chapter five).

If there is a relationship between mating success and phenotype, if mating success makes a significant contribution to *lifetime reproductive success* and if the phenotypic traits in question are *heritable*, then sexual selection may act to alter the mean value of the trait in question. In several species, other components of lifetime reproductive success have been shown to be more critical than mating success (for example, longevity in *Drosophila*, Partridge 1988). In the smooth newt more research is needed to establish the relationship between phenotypic characteristics, mating success and lifetime reproductive success. Values of lifetime reproductive success must be obtained from long-term data since short-term (seasonal) measures frequently result in both overestimation (red deer, Clutton-Brock et al. 1988; dragonfly, Fincke 1988) and underestimation (*Rana sylvatica*, Howard 1988; *Drosophila*, Partridge 1988) of the true scores.

Direct evidence for the heritability of phenotypic traits associated with mating success is also difficult to obtain and there has been a lack of research into this area in amphibians (Halliday and Tejedo 1995). One way of assessing heritability is to calculate the repeatability of traits associated with mating since only those which show consistency within individuals

can be inherited (Boake 1989). Michalak (1996) found that, with the exception of the number of fanning bouts per male, the repeatability of sexual behaviour in male and female *Triturus montandoni* was close to zero and concluded that there was little evidence for additive genetic variation in mating behaviour.

What effect might fluctuations in the operational sex ratio (demonstrated in the model) have on the operation of sexual selection in the smooth newt? Variation in the OSR and in the amount of competition for mates will *enhance or constrain the intensity and direction of sexual selection within and across generations*. For example, in a review of the association between body-size and mating success in anurans, Halliday and Tejedo (1995) found a consistent trend in the action of directional selection in only 16 of the 28 studies which spanned more than one breeding season. The remaining 12 studies exhibited variation in the intensity of selection on body size. McLain et al. (1993) found an increase in the intensity of sexual selection on the body size of the bug *Margus obscurator* in the second part of their breeding season when host plant abundance and the proportion of females in the population had declined, and emphasised that the intensity of selection may vary in response to changing ecological conditions. Madsen and Shine (1993) measured the OSR and the intensity of sexual selection on male body size in adders (*Vipera berus*) over a seven-year period. The OSR varied considerably between years and was significantly correlated with the intensity of directional selection acting on male body size. The authors emphasised the need to treat data from short-term studies with caution.

It is thus necessary to ascertain the extent to which the short-term influence of environmental conditions on breeding success obscures the more permanent effects of phenotype (Clutton-Brock 1988). This is not easy to answer and again long-term studies are essential.

As a result of the difficulties in determining the cause of observed variance in mating success, Sutherland (1985a; 1985b; 1987) goes so far as to suggest that measuring variance in mating success is not a useful method of assessing the strength of sexual selection. He proposes instead the use of search-time models in which the time spent *seeking* mates relative to the total time devoted to mating (including mate guarding, parental care, gamete replacement) is used to predict the conditions under which sexual selection may act. If the time spent searching for mates is long, then an individual with a high 'aptitude for encounter' (bright plumage, fighting ability etc.) will gain significantly more mates and sexual selection will be strong. If the time spent searching for mates is short, an increase in encounter aptitude will have little effect on mating success and sexual selection will be weak (Sutherland 1985a; 1985b; 1987). There are similarities between this approach and that of relative reproductive rate which is often measured by 'time out' during which each sex is unable to mate again (Kvarnemo and Ahnesjö 1996). The sex which has the higher reproductive rate will have more time available to search for additional mates and is likely to experience stronger sexual selection.

In the introduction, I suggested that one important justification for using a model to measure the operational sex ratio was the difficulty of obtaining accurate measurements in the field. Data from the model have clearly demonstrated the complexity of interaction between physiological and ecological factors which determine the OSR. The empirical measurement of the OSR continues to present a number of problems. For example, it is not always easy to determine which individuals are ready to mate (Kvarnemo and Ahnesjö 1996). Many studies calculate the OSR from measuring the sex ratio around focal individuals (Colwell and Oring 1988; Balshine-Earn 1996). While this may be appropriate for some mating systems, for example anuran choruses or leks (Sullivan 1986), it makes the assumption that all individuals are present for the purpose of mating and are capable of competing (Clutton-Brock and Parker

1992). Other studies calculate the OSR indirectly from knowledge of reproductive rate, often requiring additional laboratory experiments (Kvarnemo and Ahnesjö 1996) or the use of a mathematical model (Clutton-Brock and Parker 1992).

By definition, the OSR is a fluid, dynamic measurement, changing constantly as individuals become receptive or unreceptive to mating. It is therefore difficult to determine the appropriate temporal and spatial scale on which to calculate the OSR, and to compare results from studies which have used different scales. Nevertheless, there remains considerable enthusiasm for the use of the OSR, in particular in relation to variation in the quality of mates and mate choice (Kvarnemo and Ahnesjö 1996). A modelling approach, combined with empirical measurement should provide the way forward.

7.5.4 Conclusions

There are a number of ways in which the model needs to be improved and made more realistic. In particular, *females per male* and *courtships per female* are unsatisfactory measures of mating success, since the difference between deposition rate and insemination success is not taken into account. There is also considerable scope for including more aspects of female behaviour, such as additional matings following the onset of egg-laying, proceptive behaviour and sexual interference.

In the present study, the model was used to demonstrate that, in the smooth newt, fluctuations in the operational sex ratio and in the variance in mating success may occur as a result of variation in breeding sex ratio, reproductive rate and breeding synchrony both within and between seasons. Chapter six discussed the extent to which these factors are influenced by environmental conditions and life history variables. The model has thus far dealt with the potential *environmental* component of variation in mating success. For the sake of simplicity and in order to provide baseline data it was necessary to begin with individuals of equal competitive ability.

The next step is to introduce differences between individuals in order to examine the *phenotypic* component of mating success. A number of approaches could be made in this respect. For example, individual differences in reproductive rate could be introduced by varying spermatophore production or the time taken to replenish the spermatophore supply. Males could also differ in their attractiveness to females (simulating female choice for crest height, for example). Finally, the model is ideally suited to implementing an encounter-rate approach (Sutherland 1987; Arnold and Duvall 1994) in which search and handling times are the key variables determining mating success. This is particularly appropriate for species in which there is no parental care and would incorporate differences between males in their ability to find receptive females. Eventually the model could be used to estimate the relative contribution of environmental, random and phenotypic elements to the variation in mating success.

Chapter eight

Final discussion

8.1 Laboratory experiment to field observation

There are many aspects of smooth newt reproductive behaviour which are incompletely described because of the difficulties of studying these animals in their natural habitat. This thesis has taken a multi-faceted approach to study the effect of environmental and social factors on the courtship and mating dynamics of *Triturus v. vulgaris*, incorporating laboratory experiments with pairs and groups of newts, an individual-based model, a semi-natural study and field data. There were advantages and disadvantages to each approach.

In the laboratory, animals in experimental and control conditions could be watched simultaneously and were always clearly visible. Laboratory experiments, however, suffer from two potential drawbacks: the results obtained may be irrelevant to wild populations or represent artefacts of the experimental situation. Both problems were encountered during the present study. For example, the optimal temperature for spermatophore transfer (obtained in the laboratory) occurs at a time within the season when females are unlikely to be receptive (see below). Two frequently encountered artefacts were an increase in the number of sequences per courtship and a high percentage pick-up, possibly consequences of the confined space within the aquaria. The group experiments permitted investigation of some of the more complex interactions during newt courtship but inevitably increased problems of interpretation by failing to eliminate all confounding variables.

The use of a model was essential in order to study the operational sex ratio, a variable which cannot be manipulated in this species. The modelling approach helped to clarify the theoretical relationship between sex ratio, the temporal distribution of the sexes, reproductive rate and the operational sex ratio and pinpointed appropriate areas for future research. At

times, however, the model data were difficult to interpret in the context of a natural population.

The use of a semi-natural population was highly successful. It permitted a longitudinal study to be carried out in a relatively realistic environment (with the possible exception of the clarity of the water) in which individuals were undisturbed for the entire season. Individuals could be recognised by their markings, allowing measurement of mating success in smooth newts for the first time. I would recommend the use of semi-natural populations in future studies, in particular to validate the relationship between immigration, the OSR and mating success, predicted by the model. However, although it would be ideal to use artificial ponds for experiment as well as observation, it is important to bear in mind that there will be a trade-off between the number of conditions and effective sampling.

The field data provided information about the arrival patterns of smooth newts and produced questions which formed the basis for the construction of the model. There are no drawbacks to such field data, apart from their limited nature!

8.2 The influence of environmental and social factors on the courtship and mating dynamics of the smooth newt

8.2.1 Female receptivity

This thesis began with an investigation into the effect of the immediate environment (water temperature and dissolved-oxygen availability) on courtship behaviour (Chapter two). There appears to be a non-linear relationship between temperature and physiological capabilities so that display rate and the length of time for which courtship can be sustained underwater change markedly between about 8 and 13°C. The proposed optimal temperature for spermatophore transfer is just below 13°C, where the rate of deposition is still high but oxygen supply has not yet become a limiting factor.

The study of a semi-natural population (Chapter three) also showed that the frequency with which males attempt to engage females in courtship is correlated with temperature, in particular below about 10°C. However, the spermatophore transfer phase was reached in only 6% of all encounters containing display and these courtships appear to be controlled primarily by the temporal pattern of *female receptivity*. In the absence of a method of constraining the female (such as amplexus), male smooth newts cannot proceed beyond the first stages of courtship if the female is unresponsive. Females are highly receptive at the beginning of the season, probably mating two or three times in quick succession (as suggested by Hosie 1992). Additional matings occur sporadically throughout the remainder of the season, mostly after the onset of the egg-laying period. As a result, a large proportion (approximately 40%) of courtships take place at the beginning of the season when temperatures are cold (although there is a minimum threshold for deposition of about 6°C) and *there are few opportunities for males to court at the mid-season optimal temperatures.*

Although the pattern of female receptivity is crucial in determining the timing of mating within the breeding season, both proximate and ultimate mechanisms controlling receptivity are poorly understood. The lengthy egg-laying process (Baker 1992a) and the risk of pond drying have been put forward as adaptive explanations for the high level of female receptivity at the beginning of the season (Halliday pers. comm.). However, the rate of egg-laying is sensitive to temperature (Hosie 1992; Chapter three) and will therefore be slow during the cold, early spring.

As discussed in Chapter one, there are several ways in which multiple mating by females may be adaptive. At the beginning of the season, female smooth newts appear to need exposure to more than one courtship in order to induce ovulation (Waights 1998). Later in the season, females probably re-mate in order to replenish their sperm supplies (Pecio 1992, Waights, 1998 #499) and, at the same time, may attempt to gain a better mate (Gabor and Halliday 1997).

Thornhill and Alcock (1983) distinguished three types of multiple mating patterns in insects: a restricted bout of several copulations; cyclical receptivity or continuous receptivity. Species exhibiting cyclical receptivity alternate between periods of receptivity and sexually inactive periods and a variety of mechanisms were proposed to account for the reduction in receptivity. These included stimulation by the courting male, filling of the sperm storage organ and acquisition of a spermatophore. Female smooth newts fall between the first two categories with an intense bout of mating followed by irregular cyclical receptivity. The number of sperm in the spermathecae is also a possible causal mechanism of female receptivity in smooth newts although more research is needed in this area.

The pattern of female receptivity thus determines the *timing* of successful courtships within the breeding season and, since this pattern is highly irregular, there will be temporal variation in the potential for sexual selection within each season (Verrell and McCabe 1988). Smooth newts appear to provide a good example of a species in which females play a crucial role in determining mating patterns within a population, in particular via physiological and ecological constraints on the location and timing of mating (Ahnesjö et al. 1993).

8.2.2 The operational sex ratio

If the pattern of female receptivity determines the timing of mating within the season, it is a complex set of interactions between other environmental, physiological, behavioural and morphological factors which determines individual mating success (Figure 8.1). At the centre is the operational sex ratio which, in addition to female receptivity, is determined by male spermatophore production and immigration patterns.

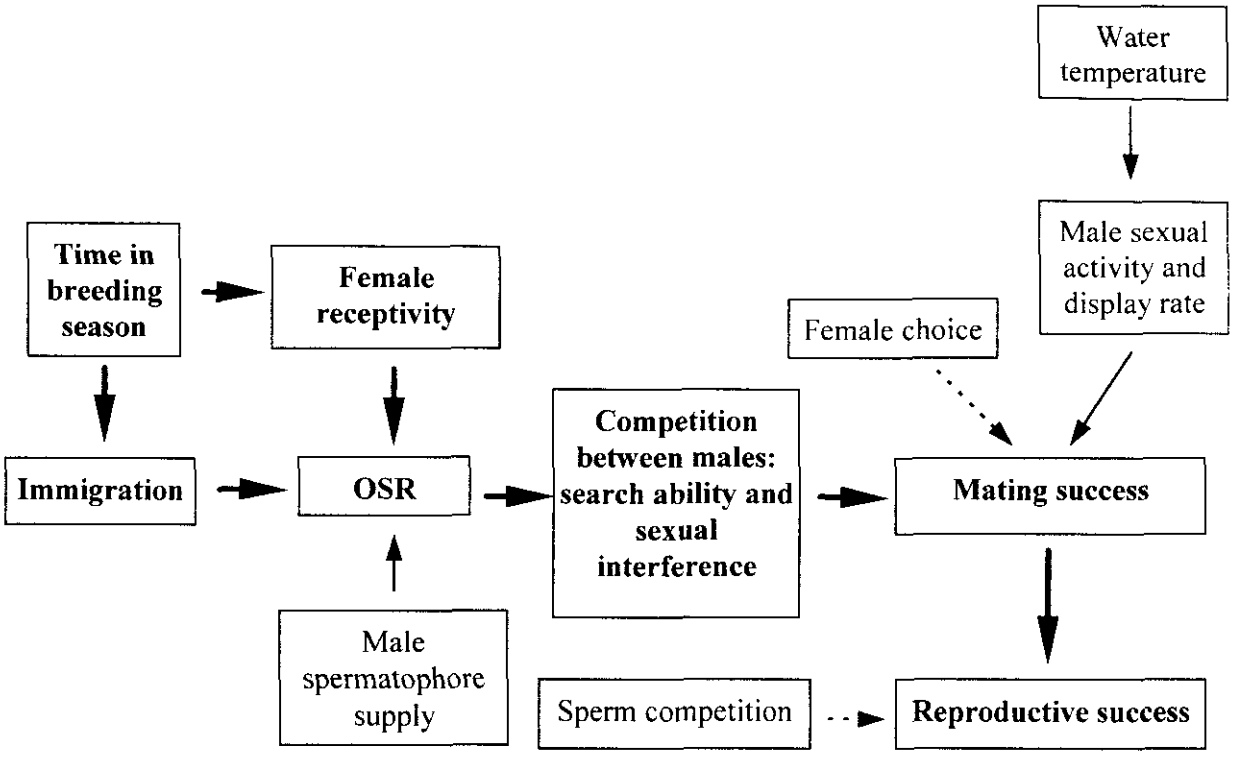


Figure 8.1. Factors affecting the courtship and mating dynamics of the smooth newt. Bold indicates a stronger link. Dotted arrow indicates that more information is needed.

Results from the model (Chapter seven) suggest that the relationship between sex ratio, the temporal distribution of mates, the OSR and competition for mates in the smooth newt generally adheres to the predictions of Emlen and Oring (1977), although other models have demonstrated less straightforward associations (Sullivan et al. 1995). During synchronous arrival, male and female mating success depends on the relative rate at which male spermatophores and female mating opportunities are used up. This, in turn, is closely related to the breeding sex ratio: competition between males and variance in male mating success increasing with an increasingly male-biased breeding sex ratio. During asynchronous arrival, males have the opportunity to renew their spermatophore supply and return to the pool of sexually active individuals. The result is a rapid increase in male bias, even within the female-biased breeding sex ratios, and greater variance in male mating success. Males arriving later experience a more competitive environment.

Halliday (1976) and Verrell (1986b) demonstrated that, in males tested to sexual exhaustion, spermatophore production is reduced on a seasonal and daily basis and it has since been assumed that the finite spermatophore supply imposes a physiological constraint on the mating capacity of males (Halliday 1998). The model too indicated that, within female-biased breeding sex ratios, males could be limited in the short-term by spermatophore availability. However (in the absence of interference) model males deposited three spermatophores per encounter, a figure closer to the maximum than to the average value for field data. Data from semi-natural and field studies strongly suggest that the *opportunities for deposition rarely exceed physiological capabilities* (Waights 1998; Chapter four) either in the long- or the short-term and, for most of the time, all breeding males in the pond will have the potential to contribute to the operational sex ratio.

At the beginning of the season, the operational sex ratio is influenced primarily by immigration patterns and will fluctuate on a daily basis as varying numbers of males and females arrive at the pond. As indicated above, however (and contrary to Verrell and McCabe 1988) I suggest that such variation will usually be in terms of the *strength of male bias*: only if the sexes arrive together, over a short period and the breeding sex ratio is female-biased, will a female-biased operational sex ratio persist for any length of time. Nevertheless, within a heterogeneous pond environment, there may be local areas in which temporary female biases arise. Under these conditions, interference between females and male preference for large females may be observed.

Towards the end of the arrival period and as more females start egg-laying, the operational sex ratio is principally determined by female receptivity. At this stage (as put forward by Verrell and McCabe 1988) there will be a consistent and strongly male-biased operational sex ratio. Within male-biased sex ratios, males are expected to compete for females and females have the opportunity to 'choose' between mates (see below). Male preferences for large

females are unlikely to be expressed because the benefits are vastly outweighed by the costs of rejecting a female (Johnstone et al. 1996).

8.2.3 Social interactions

Sexual behaviour tends to create conflict, both between members of the same sex (usually over access to mates) and between members of the opposite sex (over the outcome of mating) (Halliday 1981; Huntingford and Turner 1987). Male competition for mates in the smooth newt can be considered within the framework provided by Andersson (1994). He listed five mechanisms of competition for mates: endurance rivalry, scrambles, contests, mate choice and sperm competition. Mate choice and sperm competition are considered below. In principle, endurance rivalry between males may exist if males differ in their ability to maintain their spermatophore supply for the entire breeding season. However (as discussed above) most males are unlikely to reach their physiological limits. Scrambles and contests form part of a continuum of responses to conflicts over resources (Huntingford and Turner 1987), ranging from *non-aggressive scramble competition*, in which individuals become increasingly efficient at locating the resource through *meddling* with the activities of rivals and *manipulating* the behaviour of other individuals, to *physical coercion*. Male smooth newts engage in scramble competition (searching for receptive females), meddle with and manipulate the behaviour of rivals (sexual interference) but do not engage in physical coercion.

Thus within the constraints imposed by the social environment (the operational sex ratio and population density), the mating success of a male smooth newt will be influenced by his ability to find receptive females and to compete through sexual interference (Figure 8.1). The means by which males find receptive females has received little attention but data from the model suggest that the encounter rate of receptive females may be particularly crucial during asynchronous arrival when there are few receptive females at any given moment. It would clearly be advantageous for males to be able to distinguish between receptive and unreceptive individuals.

At the beginning of the season, unmated females are more or less synonymous with receptive females. Data from Chapter five indicate that males can recognise unmated females to whom they display preferentially. Males also display more towards unfamiliar than familiar mated females. Later in the season when female receptivity is apparently unpredictable, an unfamiliar female may represent a higher probability of insemination success than a female which has already been courted unsuccessfully. Despite this, males appear to waste considerable time chasing both unreceptive females and other males. The extent to which males vary in their ability to detect receptive females is not known but selection should favour males which are able to target receptive females more accurately (Thornhill and Alcock 1983).

Females may also benefit from receptivity advertisement. As pointed out by Thornhill and Alcock (1983) one of the costs of multiple mating is the time required to repel unwanted suitors during intermediate states of receptivity. The pursuit of apparently unreceptive females by males may constitute harassment. Clutton-Brock and Parker (1995) described harassment in terms of 'individual males that locate potentially receptive females, court and attempt to mate until the female either leaves or copulates with them.' In many species harassment is confined to receptive females and may be countered by hiding the state of receptivity (Clutton-Brock and Parker 1995). In smooth newts, however, males pursue females which frequently remain unreceptive. An unambiguous display of receptivity would allow males to avoid these females.

A male will frequently encounter a courting pair and must decide whether to interfere with the courtship or attempt to find an unattended receptive female. During interference, the efficiency of pick-up is reduced but courting males maintain a greater insemination success than interfering males. Nevertheless, the prevalence of sexual interference during the later stages of courtship in the semi-natural population is very high (50% of all courtships reaching deposition) and suggests that males are attracted towards the pheromones produced during retreat display and spermatophore transfer by other males (Belvedere et al. 1988). Since the chances of finding another female will be dependent on the operational sex ratio and

population density, it would be interesting to establish whether males alter their behaviour accordingly.

Why do males expend considerable energy courting unreceptive females and frequently engage in low-gain strategies (sexual interference)? One crucial point to emerge from this thesis (and most field studies, Verrell 1989a) is that males experience very few opportunities to deposit spermatophores over the season. In the absence of complete certainty that females are unreceptive and with a probability of insemination during interference greater than zero, males are likely to adopt a 'deposit at every opportunity' strategy, devoting the maximum possible time to pursuit, courtship and interference.

During the early stages of courtship, females find interference aversive and attempt to flee (Table 4.8, Chapter four). As courtship progresses, however, females are less easily deterred. An important question for future research is whether male sexual interference is beneficial or costly to females: whether, for example, it provides an opportunity to mate with a higher quality male or prevents insemination by a preferred male. The answer to this question will have some intriguing ramifications. If interference is advantageous to the female, selection may be expected to encourage asynchronous female arrival and receptivity advertisement. Conversely, if interference is disadvantageous, there should be selection for synchronous arrival and for reproductive state to remain cryptic (Halliday 1981). In boat-tailed grackles, the asynchronous pattern of female receptivity has been interpreted as an adaptation which encourages males to compete by establishing dominance hierarchies (Poston 1997).

8.2.4 The operation of sexual selection

Sexual selection is commonly invoked to explain the evolution of sexually dimorphic traits (Clutton-Brock 1988). In the case of the smooth newt, it is tempting to look for evidence to demonstrate that sexual selection is responsible for the evolution of male crests (Halliday 1992). Data from the artificial pond revealed variance in male mating success (although the

range in mate number was not high) and thus the opportunity for sexual selection exists. Measures of variance in mating success, however, only provide information about selection in progress and may have nothing to do with adaptation, the product of selection in the past (Grafen 1988). It is possible, therefore, to ask whether sexual selection is currently operating on male secondary sexual characteristics in the smooth newt and the answer to this question lies in the extent to which the variance in mating success is correlated with genetically controlled male traits as opposed to random or environmental factors.

The immigration patterns of smooth newts (breeding sex ratio, duration of the arrival period and extent to which males arrive before females) are highly variable and are likely to be determined by a combination of environmental and life history factors (Chapter six). More research is needed in this area, in particular to elucidate the factors which select for the timing of migration in both sexes. The model (in which males had equal competitive ability) demonstrated that this variation in immigration patterns, theoretically, has a significant impact on the operational sex ratio and competition for mates, thereby providing an environmental input to the variance in mating success (Chapter seven). In a more natural situation, however, males will differ in their competitive ability. If there is a causal relationship between heritable male traits and mating success, sexual selection may operate on those male traits. Under these circumstances, variation in the OSR will enhance or constrain the intensity of sexual selection.

Although the strength of the relationship between male characteristics and mating success has not been fully determined, there is some evidence from laboratory experiments (reviewed in Chapter four) that females prefer higher-crested males. Mate choice is the fourth mechanism of competition for mates (Andersson 1994). Traditionally, mate choice has been divided into active choice which entails sampling and selecting prospective mates and passive choice in which females are attracted to the most conspicuous mate (Parker 1983; Real 1990; Gibson and Langen 1996). In these terms, female smooth newts do not appear able to engage in active choice but may respond differentially to male morphology or behaviour (passive choice).

Several authors, however, believe that the distinction between active and passive choice is not helpful since it confuses proximate and ultimate causation (Ryan 1990; Andersson 1994). Wiley and Poston (1996) have recently proposed that the term mate choice should be broadened to include any behaviour that results in a restriction of an individual's potential set of mates. They distinguish between direct choice, which incorporates active and passive choice and requires a differential response to potential mates, and indirect choice which requires no discriminatory ability but includes any behaviour that results in non-random mating by setting the conditions for competition between members of the opposite sex. Indirect choice thus provides a formal definition for the argument put forward by Halliday (1983), and now widely accepted (Andersson 1994), that mate choice and mating competition are not mutually exclusive means of producing variance in mating success. Within this definition, fertility advertisement and asynchronous breeding in female smooth newts, which result in increased competition between males, would be examples of indirect mate choice.

Halliday (1992) proposed a causal model of (direct) female choice in the smooth newt which consists of three essential features: a spontaneous change in female receptivity over time, a threshold in receptivity at which mating occurs and the capacity for male sexual behaviour to increase female receptivity. Although Halliday envisaged female receptivity to increase linearly with time, the model is equally valid with respect to the pattern of female receptivity described in this thesis.

At the start of the season, females are highly receptive and a minimum of male display is needed to reach the threshold level for mating. As a result (and contrary to Sullivan et al. 1995) I suggest that an intense period of mating takes place both at low temperatures and before male crests are fully developed. Later in the season, female receptivity again increases spontaneously but may not reach such a high level. The stimulation required from males is greater than before and there is more potential for males to differ in their ability to stimulate females. As a result, there may be a stronger relationship between male traits and mating

success. For example, temperature and therefore male display rate will generally be higher during this part of the season and males which display at the fastest rate *within* a given temperature may be more successful. Likewise, the dorsal crest will be fully developed.

It is not known if any male characteristics are consistently associated with mating success during sexual interference. In the laboratory, male body size had no effect on the outcome of interference and the factor most relevant to pick-up appeared to be the degree to which rivals were temporarily diverted from the female (Chapter five). If there is no relationship between phenotype and the outcome of sexual interference, there will be a reduction in the intensity of selection on characters, since 'unattractive' males will gain inseminations and weaken the correlation between attractiveness and fitness. This has been described with respect to the 'sneaky' tactics of satellite males in frog choruses (Arak 1988).

Male mating success is ultimately determined by whether the female picks up the spermatophore and whether his sperm are used to fertilise her eggs. Although I have frequently referred to pick-up success as a population variable, there was marked variation between *individuals* in the relationship between the number of spermatophores elicited and the number picked up. As Halliday (1977a) pointed out twenty years ago, there is no completely satisfactory explanation to account for the success or failure of spermatophore transfer. Experimental evidence supports the idea that females pick up or miss spermatophores through choice (Green 1991a; Waights 1996) but why, for example, should females pick up some spermatophores within an encounter with the same male but not others? According to results from Chapter two, this is not associated with the position of the sequence within the encounter as was previously suggested (Halliday 1983; Hosie 1992). During interference, a female preference for certain spermatophores is likely to be frustrated when females are physically pushed away from the spermatophore.

Another hypothesis for the failure to pick up is simply accident. No relationship was found between pick-up success and the time taken from creep to deposition in the laboratory (Chapter two), suggesting that accidental failure to pick up is not directly related to the speed of display. However, the reduction in pick-up success between the laboratory, semi-natural conditions and the field indicates that the complexity of the environment may affect pick-up, increasing the likelihood that spermatophores are missed through accident. More research is needed to identify causal and functional mechanisms which determine pick-up in female smooth newts and it is likely that no single explanation will emerge.

Finally, the relationship between mating success and reproductive success in the smooth newt is not straightforward but will depend on the outcome of sperm competition (Andersson's fifth mechanism of competition for mates). Current investigation of sperm competition in smooth newts will help to elucidate many vital questions. For example, how is male paternity affected by female multiple mating over an evening, after a few days or after several weeks? Halliday (1998) proposed that, within the genus *Triturus*, females may use a combination of multiple mating and sperm competition as an adaptive strategy to increase the genetic quality of their offspring.

The question of whether sexual selection is currently operating on male secondary sexual characteristics in the smooth newt cannot be answered in full. Future research needs to focus on the link between male phenotype (crest height and other traits) and mating success in the presence of rival males and the effect of sperm competition on paternity. Equally important are long-term studies to establish the relative contributions of environmental and phenotypic factors to lifetime reproductive success over successive generations.

8.3 Conclusions

The courtship and mating dynamics of the smooth newt are determined by a complex set of interactions between physiological as well as environmental and social factors. Within the

constraints imposed by the seasonal pattern of female receptivity, there are few opportunities for courtship to take place at the optimal temperatures for spermatophore transfer, obtained in the laboratory.

The intensity of competition is affected primarily by the operational sex ratio. At the beginning of the season (when virtually all newly-arriving females are receptive) the greatest influence on the OSR is the pattern of immigration. Immigration, in turn, is likely to be controlled by climatic and life-history variables. Later in the season, the pattern of female receptivity (the control of which is largely unknown) has an overriding effect on the OSR.

Within the framework provided by the operational sex ratio, male mating success is determined by a number of social interactions including the ability to distinguish receptive females, display effectively and compete through sexual interference. There is variance in mating success among males but the relative contributions of environmental and phenotypic factors requires further clarification. Seasonal and annual variation in immigration patterns and the OSR will enhance or constrain the direction and intensity of sexual selection.

Appendix one

The software

A1.1 Object-oriented programming

Object-oriented programming (OOP) is based around a system of *objects* which can communicate with one another (Palmer 1992). Each object is defined by a set of *properties* and behaves according to a set of *rules*. The rules (known as methods in most OOP programming languages) are invoked by messages sent to the object (Bobrow and Stefik 1986). There are a number of reasons why OOP is particularly suited to simulating the natural world:-

1. Objects encapsulate information not only about their properties but also about the rules governing those properties. Objects can convey information about themselves in response to questions and, as a result, can interact in a specific way with other objects and with their environment. Each object contains a unique set of information, allowing it to act independently.

2. Objects exist within a *hierarchy*. As in taxonomy, objects at a lower level will inherit the properties and the rules governing their ancestors as well as adding new elements.

3. Polymorphism (in computer terminology) is a mechanism which allows a single rule to apply to many objects, regardless of their level within the hierarchy. Precise implementation of the rule is then specific to each object. For example the rule 'move' may apply to all objects but the method of movement could vary according to the type of object receiving the message. This permits more efficient programming.

4. Although objects may be controlled by numerous rules, each rule can be very simple. This allows accurate translation of behavioural observations into programming code.

For a clear introduction to object-oriented programming and its use in individual-based models, see Maley and Caswell (1993).

A1.2 Three-dimensional modelling

Many organisms move in three-dimensional space and yet modelling in three-dimensions is not often attempted. Each active object (newt) in the newt model is given an initial bearing, speed and angle of elevation. Changes in those variables are determined by laws which prevent collisions with other individuals, restrain the newts within the model boundaries and allow them to breathe air.

A1.3 Multi-threaded processing

Individuals think and act simultaneously. However most computer models use sequential processing in which a delay occurs as calculations are completed for every individual in turn. By the time the last individual reacts, circumstances are likely to be different from those affecting the first individual. The object-oriented approach, in which individuals communicate independently with one another, is an important step towards representing natural systems, but in many models remains constrained by serial processing (Palmer 1992). This model uses *multi-threaded processing* with each object attached to its own thread. Within each pulse of time the calculations, which provide each object with information needed to make its next decision, are distributed between the threads with no predetermined order, thus removing the deterministic element which arises from a serial process. Although objects would theoretically need to be controlled by a different processor in order to carry out instructions completely independently, this technique is conceptually much closer to reality.

There are thus five important ways in which the validity of this model is enhanced by the individual-based, object-oriented approach described above.

1. Biological processes are determined by decisions made by individuals.
2. These decisions depend on precise relationships between the individual, other individuals and the environment at a local level.

3. Highly complex behaviour is made up of numerous simple decisions.

4. Individuals make decisions simultaneously.

5. Many animals move in three dimensions.

The newt model

A1.4 Overview

The model is based on a hierarchy of objects. Each object is defined by a set of properties, some of which are inherited from objects higher in the hierarchy while others are unique to that object. Objects interact with each other according to a set of rules, the implementation of which depends on the values of the properties.

The environment object defines the space within which all other objects operate. Within this model, the term *entity* denotes all objects which exist within the pond. Entity is the highest rank within the hierarchy and contains properties common to all such objects (a name, a three-dimensional form and a location in space). Entity is divided into passive entities and active entities. Active entities have additional properties which give them the ability to move. Active entities are further divided into male and female entities with properties and rules specific to their sex. Each male and female entity is the equivalent of an individual newt. A full description of the properties is given below.

The rules govern the way in which the newts move within the model space and interact with each other. At every unit of time (set by the model clock) each active entity compares its position with that of every other entity within the model boundaries. The next decision is based on this information. The clock was set to pulse every second.

A1.5 Properties

The value for each property is assigned separately to each individual in an input file before the start of the simulation. (I used an Excel® spreadsheet). The values used in the simulations described in Chapter seven are given together with a justification where appropriate. Some

properties (such as name, sensory range, spermatophore supply decrease) remain unchanged during the simulation and these are given fixed values. Other properties (such as bearing, follow time, spermatophore supply, maximum number of courtships) change constantly as a result of interactions occurring during the simulation. These are assigned initial or maximum values.

A1.5.1 Properties associated with all entities

1. *Name*. The name identifies each entity and permits the program to return values associated with that entity. Each newt had a name.

2. *XYZ*: the location in space. Seven passive entities were placed within the pond in order to increase the complexity of the environment. These were given XY co-ordinates which distributed them across the floor of the pond in an irregular fashion. The Z co-ordinate was set at zero. The initial XY co-ordinates for the newts were assigned randomly by the computer while the Z co-ordinate (height) was set at 20mm (to ensure that the newts appeared above the bottom of the pond).

3. *Width, Depth, Height*: entity dimensions. Passive and active entities were all three-dimensional rectangular or cuboid shapes. Passive entities were given a variety of sizes, ranging from 80 × 80 × 500mm to 120 × 120 × 400mm. Newts were 45mm (long) × 8mm (across) × 10mm (high), the approximate dimensions of smooth newts before crest development.

A1.5.2 Properties associated with active entities (newts)

1. *Bearing*: the direction of movement (range 0 – 359°). The initial bearing for newts was assigned at random by the computer.

2. *Angle of elevation*: the angle at which the newts move from the bottom to the top of the pond, associated with breathing air. The initial angle of elevation was 0° (horizontal) and

the newts remained on the bottom of the pond until the breathing rule was implemented (see below).

3. *Speed*: unit distance travelled per unit time. The basic speed of movement for newts was 50mm/second. Since this was a fairly approximate estimation, taken from behavioural observations, a sensitivity analysis was carried out with the speed of movement reduced to 25mm/second (Appendix three).

4. *Sensory range*: the distance within which other newts can be perceived. This was estimated as 500mm following observation of newt behaviour within the artificial pond. Again, a sensitivity analysis was carried out to look at the effect of changing this value (Appendix three).

5. *Breathe interval*: the period after which newts ascend to the pond surface to breathe.

A1.5.3 Properties associated with newt courtship: males

1. *Follow time*: the time during which a male follows a female before discovering if she is receptive. Follow time was 80 seconds, calculated from courtships which did not lead to deposition, observed at the artificial pond (Chapter three).

2. *Follow speed*: the speed at which males move towards females. Males attracted to females increased their speed from 50mm to 80mm/second. Follow speed was estimated from observation of newts within the artificial pond.

3. *Court time 1 – 4*: the duration of each courtship period. Courtship is divided into four periods. The first period (40 seconds) represents static and retreat display during the first sequence. The second period (30 seconds) is the first spermatophore transfer phase, ending with the first deposition. Period three represents a short second sequence (50 seconds) leading to the second spermatophore deposition and period four (70 seconds) represents display and deposition during the third sequence. The court time values, obtained from analysis of courtships at the cold temperature (experiment one, Chapter two) and from the

literature (Halliday 1976) were fairly approximate. A sensitivity analysis was therefore carried out with an alternative set of court times (Appendix three).

4. *Spermatophore supply*: the initial number of spermatophores assigned to each male. This was set at eight, considered representative of males at the beginning of the season (see Appendix two).

5. *Male refractory period*: the duration of the refractory period. This was set at 10800 seconds (three hours) which represented one night. The refractory period was not used in the single night simulations.

6. *Spermatophore supply decrease*: after each refractory period, males receive a new spermatophore supply which may contain fewer spermatophores. Each new spermatophore supply contained two fewer spermatophores (see Appendix two).

A1.5.4 Properties associated with newt courtship: females

1. *Maximum number of courtships*: the number of courtships after which females are no longer receptive. For the purposes of the model, courtship was defined as an encounter resulting in at least one deposition. This was set at three. Data from the artificial pond (Chapter four) and Hosie (1992) suggested that females court two or three times at the beginning of the season, before the start of the egg-laying period.

2. *Female refractory period*: the time taken for females to become receptive again after the maximum number of courtships have been used up. During the current simulations, which were concerned with the first few days of the season only, the refractory period value was very high so that females remained unreceptive. It could, however, be used to return females to the pool of receptive individuals following a period of egg-laying.

A1.6 Rules governing active entities

A1.6.1 Movement

Newts (active entities) move anywhere within the model boundaries, a three-dimensional space representing a pond. The model space was 3000mm × 3000mm × 1500mm (depth). If an active entity reaches one side of the model space, it continues with a new bearing which forms the mirror image of the angle at which the boundary was approached. The angle of elevation remains the same. If an active entity reaches the top or bottom of the model, it continues on the same bearing with the opposite angle of elevation (for example +30° becomes -30°).

The newts are prevented from colliding with one another and with passive entities. If a newt comes within 1mm of another entity, a series of instructions follow which allow it to navigate past and resume its original bearing and angle of elevation.

A1.6.2 Breathing

Both male and female newts can breathe at a set interval of time. At the start of breathing the angle of elevation changed to 60°, unless the newt is already moving towards the surface at a greater elevation. The newt resumes its previous angle of elevation after returning to the bottom of the pond. The breathing function is given a weighting. If the weighting has a very high value, the need to breathe will override all other activities (such as being attracted towards another newt). If the weighting is set at a lower value, other activities can compete with the need to breathe. For the sake of simplicity, breathing was not incorporated into the simulations described in Chapter seven. This reflects the cold temperature conditions (Chapter two) in which there was no conflict between the need to breathe and courtship activity.

A1.7 Rules associated with courtship

A1.7.1 Attraction factor

The sensory range denotes the distance within which other entities can be perceived. If one or more newts come within the sensory range of an individual, the attraction factor decides which is most attractive using the following series of questions.

1. Do I need to breathe? As mentioned above, if breathing is given a high weighting, the need to breathe will overrule all other activities.

2. Am I already following? If yes, stay following: newts cannot be attracted towards another individual while following.

3. Am I already courting? If yes, stay courting: newts cannot be attracted towards another individual during courtship.

4. Is this the last individual I was attracted to? If yes, an attraction factor of zero is returned. (This part of the procedure prevents individuals being constantly attracted back to the same newts).

5. Compare sex.

Female newts:-	Is the other individual a male? If so, an attraction factor of zero is returned. Is the other individual a female? If so, an attraction factor of zero is returned.
Male newts:-	Is the other individual a male? If so, an attraction factor of zero is returned. Is the other individual a female? If so, a positive attraction factor is returned.

In the current model, this procedure is applied so that males are attracted to females. It would be possible, however, to assign other values to the attraction factor in order to create different degrees of attraction between all combinations of the sexes.

A1.7.2 Courtship

If a male is attracted towards a female, he increases his speed and follows her for the follow time period. After this he asks:-

1. Does the male have any spermatophores? (The male will have no spermatophores if his spermatophore supply has reached zero and he is still within the refractory period).
2. Is the female receptive? (The female will not be receptive if she has obtained the maximum number of courtships).
3. Is the female already being courted by another male?

If the answer to the third question is no and *provided the female is receptive and the male has a supply of spermatophores*, the newts stop moving and courtship begins. If the answer to the third question is yes the male will proceed, not with courtship but with *interference* (see below). At present, courtship is divided into four periods. At the end of the second, third and fourth periods, the male is asked if he has remaining spermatophores. If the answer is 'yes', deposition occurs. The maximum number of spermatophores which can be deposited per encounter is therefore three. This was chosen because the frequency distribution in number of depositions per encounter at the cold temperature (experiment one, Chapter two) peaked at three depositions (43%). Data from the artificial pond (Chapter three) also suggested that three was a reasonable maximum number of depositions per encounter. The maximum number of spermatophores could be reduced within the current model. An increase would require the addition of a fifth court period. At the end of courtship, the male loses his attraction to the female and the newts resume their original speed and bearing.

A1.7.3 Interference

The maximum number of courting males denotes the number of males which can attempt to court a female at any one time. In the current version of the model this was always set to two and the process of interference was based on a maximum of two males. In the future, it would be possible to increase the number of interfering males. The outcome of interference depends on the court time in which the interfering male first approaches the courting pair. The

encounter is always terminated at the end of the period in which interference occurs and both males lose their attraction to the female. The details of interference are as follows:-

1. Court period one: no deposition occurs. This represents the period in which females find interference aversive (Chapter five).
2. Court period two: the courting male only deposits. This results in a total of one deposition by the courting male and none by the interferer.
3. Court period three: both males deposit. The courter therefore has a total of two depositions and the interferer one.
4. Court period four: the interfering male only deposits. Again the courter has a total of two depositions and the interferer one.

This is a simple set of rules, derived from the results presented in Chapter five which showed that the probability of deposition by the interfering male was less than that of the courting male and was particularly rare during the first sequence. Males in their refractory period cannot interfere.

A1.7.4 Returned values

Each individual is programmed to store changes which take place in the values of specific properties (for example, the total number of spermatophores deposited or the total number of courting males). At any time during the simulation it is possible to save and output these values. In this way, data stored by the population can be sampled at specific times or regular intervals in much the same way as experimental or observational data are obtained.

A1.7.5 The screen

The movements of the newts can be watched using an aerial or side view of the pond. Individuals can display certain values while the model is running in order to give a clearer picture of their current state and interactions:-

Males

1. Name.
2. Follow/Court/Interfere: if the male is currently engaged in one of those activities.
3. Court Period (1 – 4).
4. Number of Females: the number of females for whom the male has deposited at least one spermatophore.
5. Spa Reserve: the number of spermatophores remaining in the spermatophore supply.
6. Spa Encounter: the number of spermatophores deposited during the present encounter.

Females

1. Name.
2. Courting Males: the number of males courting the female at any one time.
3. Number of Courtships: the number of courtships obtained by the female during which at least one spermatophore was deposited.

Males are depicted by blue and females by yellow rectangles on the screen. The colour is dulled if the newts are within their refractory periods. This was particularly useful during testing of the model, since it allowed verification that the program was running correctly. Each simulation is set up using the settings menu. 'Run time' refers to the time period over which the entire simulation is run. 'Save every' allows regular saves to be made of the returned values (see above). 'Merge every' is used for the addition of new individuals during the simulation. 'Repeat' determines the number of replicates obtained from each simulation.

The software design was implemented by David Wilcockson, using Object Pascal (Borland® 1996).

Appendix two

Spermatophore supply

A2.1 Introduction

In Chapter four, I discussed a number of experiments which showed that the finite spermatophore supply limits male smooth newt mating capacity on a short- and long-term basis. Males were encouraged to deposit to sexual exhaustion (using a female held in a strait-jacket) and were re-tested after a period of hours or days (Halliday 1976; Verrell 1986b). In the short-term, males required a recovery period of 48 hours for deposition levels to return to the previous maximum (Verrell 1986b). Data from the artificial pond, however, suggested that even at the beginning of the season, males rarely deposit more than one or two spermatophores per encounter, a much lower number than obtained using a strait-jacketed female.

I designed an experiment to compare spermatophore deposition over two and four encounters, following a high or low level of deposition during the first encounter.

A2.2 Methods

In the first part of the experiment, males were divided into two groups. Using a female held in a strait-jacket (see Chapter two for details), the first group (n = 14) were courted to sexual exhaustion (representing a high demand on their spermatophore resources). Sexual exhaustion was defined by a minimum period of three minutes following deposition, during which time the male failed to reach creep again. This was slightly more stringent than the two minute period used by Verrell (1986b). The second group of males (n = 12) were allowed to deposit two spermatophores only (representing a lower demand on their spermatophore supply). Courtship was continued, however, until the males had reached creep in the third sequence to ensure that more spermatophores were available. Following a rest period of between one and two hours, both groups of males were re-tested to sexual exhaustion.

The aim of the second part of the experiment was to see if males could sustain deposition twice a day over two consecutive days and whether the demand on spermatophore reserves during the first test had an effect the following day. The procedure was repeated with two additional groups of males and was followed by another two tests 24 hours later. Both tests on the second day were to sexual exhaustion and took place one to two hours apart. Thirteen males began with a low-demand test and twelve with a high-demand test.

The males in this experiment had been used in previous experiments during the breeding season and therefore did not represent individuals at the peak of spermatophore production. They were kept apart from females for at least four days before the start of the experiment to ensure that there were no differences in their state of recovery following previous courtships. Males were distributed so that there was a mix of sizes across each experimental group. These experiments were conducted in an outdoor hut where the newts experienced natural light and temperature conditions. The males were allowed to settle for at least 30 minutes before the start of the experiment. A total of 12 females were anaesthetised with MS222. The experiment was conducted between 19 March and 4 May, 1996.

A2.3 Analysis and results

Results from the first experiment in which males were tested twice on one day with an interval between tests of one to two hours are given in Table A.1. Low refers to a low demand on the males' spermatophore supply while high denotes a test to sexual exhaustion.

Group 1			Group 2		
Low	**High**	**Difference?**	**High**	**High**	**Difference?**
2	2	No	3	2	-
2	2	No	3	2	-
2	2	No	3	3	No
2	3	+	3	2	-
2	2	No	2	2	No
2	4	+	3	3	No
2	3	+	3	2	-
2	3	+	2	2	No
2	2	No	2	1	-
2	4	+	4	3	-
2	2	No	3	2	-
2	1	-	3	3	No
			2	2	No
			2	2	No
2	**2.5**		**2.7**	**2.2**	

Table A.1. Spermatophore deposition during two tests separated by one to two hours. Group 1: low demand in first test. Group two: high demand in first test. The mean score is given in the last row (bold).

The difference in mean number of spermatophores deposited during the second test is very small, but there is a difference between the two groups in the direction of change between the first and second tests. In the group which did not deposit to capacity during the first test (low demand), 50% of males deposited the same number, 42% deposited more and 8% deposited fewer spermatophores during the second test. In the second group (high demand), 50% again deposited the same number and 50% deposited fewer spermatophores during the second test.

The mean and total number of spermatophores deposited over the two day experiment is given in Table A.2. Males produced a mean total of 7.5 spermatophores (range 5 – 11). On day one, spermatophore production was reduced during the second test, following an initial encounter with high demand and remained stable following a low-demand test. The third test (day two) produced a slight increase in spermatophore number while spermatophore production was low in both groups by the fourth test.

	Day 1		Day 2		
Group 1	Low	High	High	High	Total
Mean	2 (± 0.00)	2 (± 0.6)	2.23 (± 0.75)	1.3 (± 0.62)	7.54 (± 1.45)
Group 2	High	High	High	High	Total
Mean	2.5 (± 0.52)	1.6 (± 0.78)	2.16 (± 0.58)	1.3 (± 0.65)	7.66 (± 1.61)

Table A.2. Mean (± SD) spermatophore deposition in four encounters over two days with a high or low demand in the first encounter.

A2.4 Discussion

These results were obtained from males which had already courted during the season and would therefore not be expected to show maximum spermatophore production. Nevertheless, all males were able to continue deposition over the four tests with a mean total of 7.5 spermatophores. These results agree with Verrell's (1986b) experiment in that they illustrate a decline in spermatophore production over successive tests with a rest period of less than 48 hours. However, a rest period of one to two hours only was sufficient to allow males to deposit again. Following an initial test to sexual exhaustion, spermatophore production during the second test was reduced by 81% in the one-day experiment and 64% in the two-day experiment, comparable with the reduction found by Verrell after 12 hours. However, following low demand during the initial test, spermatophore production increased by 25% (one-day experiment) or remained stable (two-day experiment).

It was shown in Chapter four that, in a semi-natural situation, male smooth newts rarely deposit more than one or two spermatophores during an encounter. In view of the results presented here, it is likely that males can engage in two or more encounters within an evening for up to two consecutive nights (even during the middle of the season). Contrary to Verrell (1986b) I suggest that, at the beginning of the season, males are capable of taking advantage of most opportunities which arise for multiple mating.

In this experiment, males produced an average of 7 – 8 spermatophores over a period of 24 hours. Eight was therefore chosen as the maximum spermatophore supply used in the model (see Chapter seven and Appendix one). Following eight depositions, a refractory period of 24 hours was introduced. Since eight probably represents a relatively high value, the spermatophore supply was reduced to six after the refractory period.

Appendix three

Sensitivity analyses

A series of sensitivity analyses was carried out to assess the impact of changing some of the values given to the variables described in Appendix one. All the simulations used a population of twenty individuals with a sex ratio of unity and ran for three hours. 30 replicates of each simulation were obtained.

A3.1 Court time

Since the original values of court time period probably erred on the short side, this simulation contained longer court times. Court time 1 = 100 seconds; court time 2 = 50 seconds; court time 3 = 60 seconds; court time 4 = 80 seconds.

A3.1.1 Results

The mean, standard deviation and standardised variance for the number of *courtships per female* and *females per male* and the frequency distribution of *females per male* are given in Table A.3 and Table A.4.

	Shorter court times		**Longer court times**	
	Courtships per female	***Females per male***	***Courtships per female***	***Females per male***
$\bar{x}$	3	3.32	3	3.3
SD	0	0.85	0	0.97
$\sigma^2/\bar{x}^2$	0	0.06	0	0.08

Table A.3. The mean, standard deviation and standardised variance for *courtships per male* and *females per male* from simulations with shorter and longer court times.

Females per male	**0**	**1**	**2**	**3**	**4**	**5**	**6**
Shorter court times	1	6	34	132	109	18	0
Longer court times	0	13	37	126	97	23	4

Table A.4. The frequency distribution of *females per male* from simulations with shorter and longer court times.

There was clearly no significant difference between the results after three hours of simulation (Kolmogorov-Smirnov on the frequency distribution of *females per male*: D_{mn} =

0.03, m = 300, n = 300, p > 0.1). The OSR, however, took marginally longer to become male-biased (Table A.5) with longer court times.

Minutes	0	30	60	90	120
Shorter court times	50	71.4	97	100	100
Longer court times	50	59	88	100	100

Table A.5. Changes in the OSR over time from simulations with shorter and longer court times. Ratio: (100/total receptive females + active males) × active males. 50: unity, 100: male-biased.

The median number of *courting males per female* increased significantly at the longer court times (the median test: $X^2 = 21.68$, df = 1, m = 300, n = 300, **$p < 0.001$**) suggesting a greater incidence of interference (Table A.6).

	Shorter court times	Longer court times
Median	6	7
1st quartile	5	5
3rd quartile	9	11

Table A.6. *Courting males per female* from simulations with shorter and longer court times.

A3.1.2 Discussion

The length of the court time periods had no effect on the values for the number of *females per male* and *courtships per female*, obtained at the end of the three hour simulation. However, with the longer court periods, the increase in time needed to complete courtship and the greater opportunity this gave for interference, marginally delayed the shift towards a male-biased OSR.

A3.2 Speed of movement

A simulation was carried out in which the newts moved at half the original speed (25mm/second).

A3.2.1 Results

The mean, standard deviation and standardised variance for the number of *courtships per female* and *females per male* and the frequency distribution of *females per male* are given in Table A.7 and Table A.8.

	50mm/second		25mm/second	
	Courtships per female	*Females per male*	*Courtships per female*	*Females per male*
$\bar{x}$	3	3.32	3	3.33
SD	0	0.85	0	0.84
$\sigma^2/\bar{x}^2$	0	0.06	0	0.06

Table A.7. The mean, standard deviation and standardised variance for *courtships per female* and *females per male* from simulations with two different speeds of movement.

Females per male	0	1	2	3	4	5	6
50mm/second	1	6	34	132	109	18	0
25mm/second	1	5	36	125	117	16	0

Table A.8. The frequency distribution of *females per male* from simulations with two different speeds of movement.

Speed of movement had no effect on the mean and variance in the number of *females per male* or *courtships per female* or on the frequency distribution of *females per male* (Kolmogorov-Smirnov on the frequency distribution of *females per male*: $D_{mn} = 0.02$, m = 300, n = 300, $p > 0.1$).

The OSR took marginally longer to become male-biased with a slower speed of movement (Table A.9).

Minutes	0	30	60	90	120
50mm/second	50	71.4	97	100	100
25mm/second	50	65	95	100	100

Table A.9. Changes in the OSR over time from simulations with two different speeds of movement. Ratio: (100/total receptive females + active males) × active males. 50: unity, 100: male-biased.

There was no difference between the two simulations in the number of *courting males per female* (the median test: $X^2 = 2.21$, df = 1, m = 300, n = 300, $p > 0.1$) (Table A.10).

	50mm/second	25mm/second
Median	6	6
1st quartile	5	4
3rd quartile	9	8

Table A.10. *Courting males per female* from simulations with two different speeds of movement.

A3.2.2 Discussion

There was no effect of speed of movement on the mean number of *females per male* or *courtships per female* or on the median number of *courting males per female*. The slightly longer time taken for the OSR to change reflected the extra time needed for encounters between newts to occur.

A3.3 Sensory range

An accurate measurement of the maximum sensory range of newts (both visual and olfactory) does not appear to be available in the literature. The large body of research into visual behaviour in salamanders has concentrated on the details of prey detection and feeding behaviour (Roth 1987). One experiment (Galliard 1985 in Roth 1987) suggested that the frog (*Rana esculenta*) can estimate prey distances of between 6 and 300cm. It has also been demonstrated that in water, visual acuity in *Triturus vulgaris* does not decrease over a distance of at least 14cm (Grüsser-Cornehls and Himstedt 1976) although Manteuffel and Himstedt (1978) describe aquatic *T. cristatus* as myopic.

In the context of mating behaviour, males appear able to perceive females over at least 50cm or the width of an average aquarium (von Prechtl 1951; Halliday 1974, pers. obs.) although visibility may be enhanced by clean water in experimental conditions. It is not certain whether mate location is dependent primarily on visual or olfactory cues. Cogălniceanu (1994) suggested that olfaction is more important than vision in mate location. In his experiments, Male *T. vulgaris* responded to female odours in a Y-maze test from a distance of 40cm, although the maximum distance for detection was not tested (Cogălniceanu 1992). On the other hand, Halliday (1974) noted that, within an aquarium, a direct approach by the male is usually initiated by some movement on the part of the female. Himstedt (1979) showed that the red belly colour of female *Triturus alpestris* was an important stimulus for the initiation of male courtship activity (but did not state the distance of the models from the male).

The value for sensory range (500mm) used in the previous simulations was an estimate based on my observations and sensory range was therefore doubled to 1000mm and halved to 250mm.

A3.3.1 Results

The mean, standard deviation and standardised variance for the number of *courtships per female* and *females per male* and the frequency distribution of *females per male* are given in Table A.11 and Table A.12.

	250mm		**500mm**		**1000mm**	
	Courtships per female	***Females per male***	***Courtships per female***	***Females per male***	***Courtships per female***	***Females per male***
$\bar{x}$	3	3.3	3	3.32	3	3.33
SD	0	0.88	0	0.85	0	0.95
$\sigma^2/\bar{x}^2$	0	0.07	0	0.06	0	0.88

Table A.11. The mean, standard deviation and standardised variance for *courtships per female* and *females per male* from simulations with three different sensory ranges.

Females per male	**0**	**1**	**2**	**3**	**4**	**5**	**6**
250mm	0	8	37	136	97	21	1
500mm	0	6	34	132	109	18	0
1000mm	1	9	36	126	102	22	4

Table A.12. The frequency distribution of *females per male* from simulations with three different sensory ranges.

Sensory range had no effect on the mean and variance in the number of *females per male* or *courtships per female* or on the frequency distribution of *females per male* (Kolmogorov-Smirnov on the frequency distribution of *females per male*. 250mm and 500mm: $D_{mn} = 0.26$, $m = 300$, $n = 300$, $p > 0.1$. 500mm and 1000mm: $D_{mn} = 0.26$, $m = 300$, $n = 300$, $p > 0.1$).

The shift to a male-biased OSR occurred more rapidly with a decreasing sensory range (Table A.13).

Minutes	**0**	**30**	**60**	**90**	**120**
250mm	50	80	99	100	100
500mm	50	71.4	97	100	100
1000mm	50	61	80	98	100

Table A.13. Changes in the OSR over time from simulations with three different sensory ranges. Ratio: (100/total receptive females + active males) × active males. 50: unity, 100: male-biased.

The median number of *courting males per female* increased significantly with increasing sensory range (the extension of the median test, Siegel and Castellan 1988, p200: X2 = 29.55, df = 2, N = 900, $p < 0.001$) (Table A.14).

	250mm	500mm	1000mm
Median	5	6	7
1st quartile	4	5	5
3rd quartile	7	9	11

Table A.14. *Courting males per female* from simulations with three different sensory ranges.

A3.3.2 Discussion

The number of *females per male* and *courtships per female* was not affected by sensory range. However, with an increase in the sensory range (and thus the perception distance of the newts) the amount of competition between males (*courting males per female*) increased. This was accompanied by a delay in the time taken to shift to a male-biased OSR.

A3.4 Density

The breeding sex ratio and synchronous arrival simulations described in sections 7.4.1 and 7.4.3 of Chapter seven differed only in population density (a total of 20 or 40 individuals). These simulations were compared directly to establish the effect of a change in density on the number of *females per male*, *courtships per female*, *courting males per female* and the OSR. Results were available at three sex ratio; Fbias (8m:12f or 16m:24f), unity (10m:10f or 20m:20f) and Mbias (12m:8f or 24m:16f).

A3.4.1 Results

The mean, standard deviation and standardised variance for the number of *courtships per female* and *females per male* are given in Table A.11. Since the sample sizes differ between the simulations, Table A.12 shows the percentage frequency distribution of *females per male*.

		Fbias		Unity		Mbias	
		Courtships per female	*Females per male*	*Courtships per female*	*Females per male*	*Courtships per female*	*Females per male*
20	$\bar{x}$	2.2	3.6	3	3.38	3	2.28
	SD	0.9	0.65	0	1.02	0	1.17
	$\sigma^2/\bar{x}^2$	0.16	0.03	0	0.09	0	0.26
40	$\bar{x}$	2.3	3.73	3	3.3	3	2.27
	SD	0.94	0.68	0	0.99	0	1.21
	$\sigma^2/\bar{x}^2$	0.16	0.03	0	0.09	0	0.28

Table A.15. The mean, standard deviation and standardised variance for *courtships per female* and *females per male* from simulations with different population densities (20 and 40 individuals).

Density	*Females per male*	0	1	2	3	4	5	6
20	**Fbias**	0	0	0	47.91	44.16	7.08	0.83
	Unity	0.33	5	11	35	38.33	9.33	1
	Mbias	5.27	20.55	34.16	22.5	15	2.5	0
40	**Fbias**	0	0	0	39.16	50	9.37	1.45
	Unity	1.16	3	13.16	38	36.66	7.16	0.83
	Mbias	6.66	21.97	30.27	25.27	14.16	2.22	0.41

Table A.16. Percentage frequency distribution of *females per male* from simulations with different population densities (20 and 40 individuals).

Density had no effect on the mean and variance in the number of *females per male* or *courtships per female* or on the frequency distribution of *females per male* at any sex ratio. (Kolmogorov-Smirnov tests on the frequency distribution of *females per male*. Female-bias: $D_{mn} = 0.09$, m = 240, n = 480, $p > 0.1$. Unity: $D_{mn} = 0.03$, m = 300, n = 600, $p > 0.1$. Male bias: $D_{mn} = 0.03$, m = 360, n = 720, $p > 0.1$).

Data on the changes in OSR over time were only available for the population at unity. A slightly longer time was taken for the OSR to become fully male-biased at the higher density (Table A.17).

Density	0	30	60	90	120
20	50	71.4	97	100	100
40	50	66.6	91.8	99	100

Table A.17. Changes in the OSR over time from simulations with different population densities (20 and 40 individuals). Ratio: (100/total receptive females + active males) × active males. 50: unity, 100: male-biased.

There was no difference between densities in the median number of *courting males per female* (Table A.18). (The median test. Female bias: $X^2 = 1.2$, df = 1, m = 360, n = 720, p > 0.2. Unity: $X^2 = 0.01$, df = 1, m = 300, n = 600, p > 0.9. Male bias: $X^2 = 0.51$, df = 1, m = 240, n = 480, p > 0.3).

Density		Fbias	Unity	Mbias
20	**Median**	3	6	8
	1st quartile	3	4	6
	3rd quartile	5	8.25	11
40	**Median**	3.5	6	8
	1st quartile	2	4	5
	3rd quartile	6	9	11

Table A.18. *Courting males per female* from simulations with different population densities (20 and 40 individuals).

A3.4.2 Discussion

An increase in density from 20 to 40 individuals had no significant effect on any of the measurements taken during these simulations.

A3.5 General discussion

None of the changes in value of the above properties affected the results obtained for the number of *females per male* or *courtships per female* at the end of the three hour simulation. The longer court times and longer sensory range were accompanied by an increase in the number of *courting males per female* and an increase in the time taken to develop a male-biased OSR, but in neither case was this sufficient to alter the variance in *females per male* to a significant degree. Nevertheless, this shows that court time and sensory range are relatively sensitive to changes in value and care should be taken to obtain accurate figures for these properties. Population density had no effect on the results obtained during these simulations, confirming that it is the sex ratio, rather than density which is of prime importance in determining the nature of competition between individuals. If the density was altered more dramatically, however, the time taken for encounters to occur and therefore the time taken for the OSR to become limiting could be expected to change more significantly.

Overall, the changes seen as a result of these sensitivity analyses were on a much smaller scale than those seen during the simulations investigating sex ratio, reproductive rate and arrival patterns, described in Chapter seven. The model can thus be described as robust in relation to these 'background' properties.

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